

Introduction

The use of Traditional Chinese Medicines (TCM) has a long history in China, Korea, Japan and other Asian countries. Currently more TCM crops than ever are being cultivated in large scale farming operations where agrochemicals are in use. The routine screening and quantitation for hundreds of pesticides plays an important role to increase the safety of herbal drug products. Contamination with noxious weeds or a fungal affection has raised the importance for an additional testing for other common contaminants such as mycotoxins and pyrrolizidine alkaloids ^{ref 1,2}.

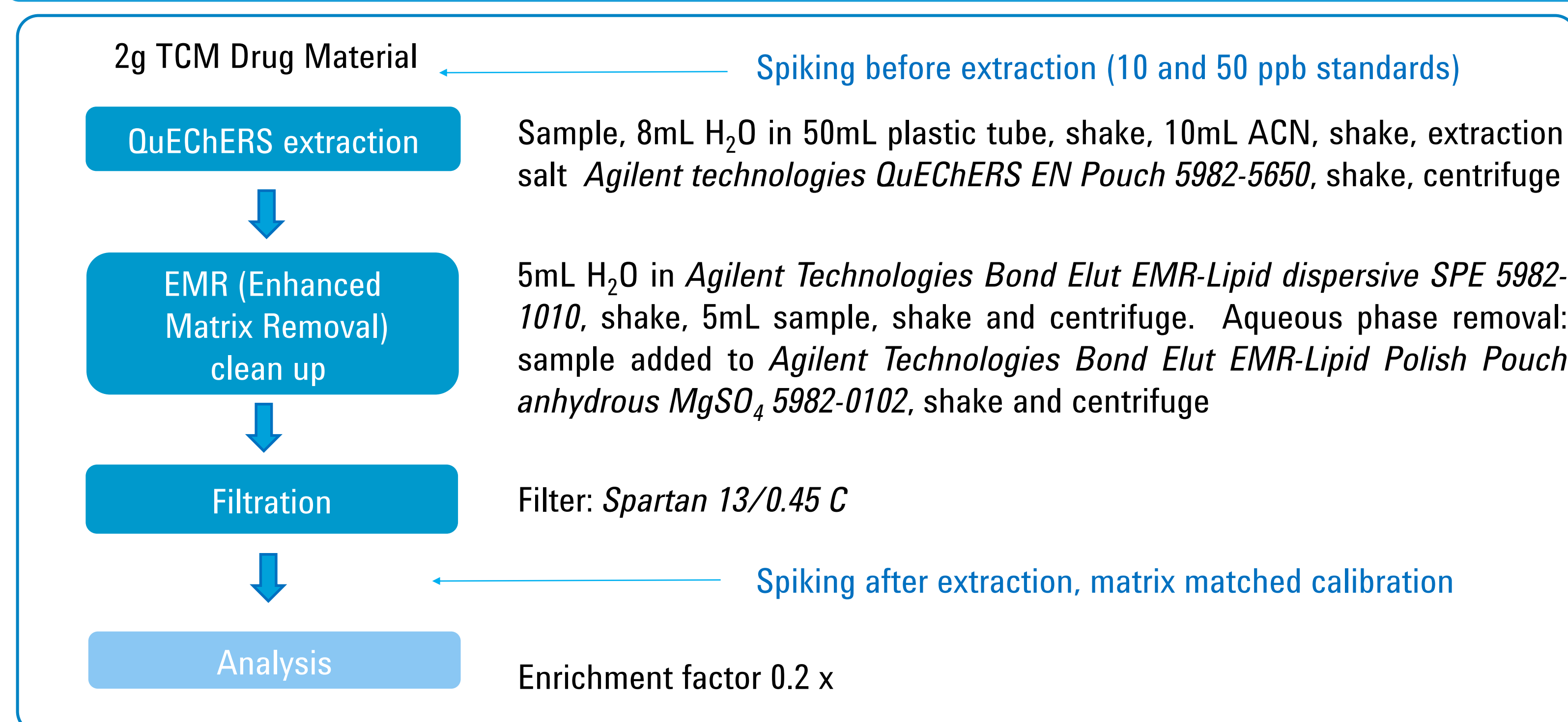


Table 1. A total of 10 TCM drugs have been investigated

Herba epimedii	Folium bambusae
Cinamoni ramulus	Glycyrrhizae radix
Fructus gardenia	Poriae sclerotium cocos alba
Flos magnoliae	Radix ginseng
Mori folium	Leonuri herba

In this poster we demonstrate a new workflow for a combined screening and quantification of pesticides, mycotoxins and pyrrolizidine alkaloids. It contains a modified QuEChERS extraction process, a UHPLC separation and a highly sensitive detection using a triple quadrupole (QQQ) mass spectrometer operated with dynamic MRM and fast polarity switching ^{ref 3}.

Experimental



A robust cleanup process is required to stay within the recovery specifications for all contaminants covering a broad polarity range. A simple QuEChERS extraction first showed non satisfying results for some of the herbal drug samples due to strong matrix effects. Therefore, an EMR (Enhanced Matrix Removal) step was added to generate more confident results.

Results and Discussion

Method performance

The quantitation was based on standard additions or matrix matched calibrations to compensate matrix effects during electrospray ionization. Linear calibration curves were obtained for the majority of compounds in the relevant concentration range of 0.1 to 100 µg/kg in water and 0.5 to 100 µg/kg in matrix (Mori folium). Method performance characteristics are shown in Table 3.

Table 3. Performance characteristics for selected substances (n.a.=not assessed, n.e.=not existing)

Analyte	In solvent (water)		In Mori folium extract		European legislation ^{ref 4} in leaves and herbs (in µg/kg)
	LOD (in µg/kg)	LOQ (in µg/kg)	LOD (in µg/kg)	LOQ (in µg/kg)	
Pesticides					
Buprofezin	<0.1	0.1	n.a.	<2ppb	50
Carbendazim	<0.1	0.5	n.a.	n.a.	100
MCPA	2	10	10	25	100
Profenofos	0.1	0.5	<0.5	2.5	50
Propargite	0.1	0.5	5	10	50
Triflumuron	0.1	0.5	<0.5	2.5	50
Pyrrolizidine alkaloids					
Europine	<0.1	0.1	<0.5	0.5	n.e.
Lasiocarpine	<0.1	0.5	25	25	n.e.
Mycotoxins					
Aflatoxin B1	<0.1	0.5	2.5	5	n.e.
Ochratoxin	1	2	10	25	n.e.

For recovery studies three TCM drugs were spiked with standard compounds before and after extraction at different concentration levels and analyzed by using the described method above. The sample preparation protocol allowed an efficient extraction according to SANTE guidelines^{ref 5} of a representative number of standard compounds even after adding the EMR cleanup step. Figure 3 shows that 82% of the standard compounds have recoveries over 70%.

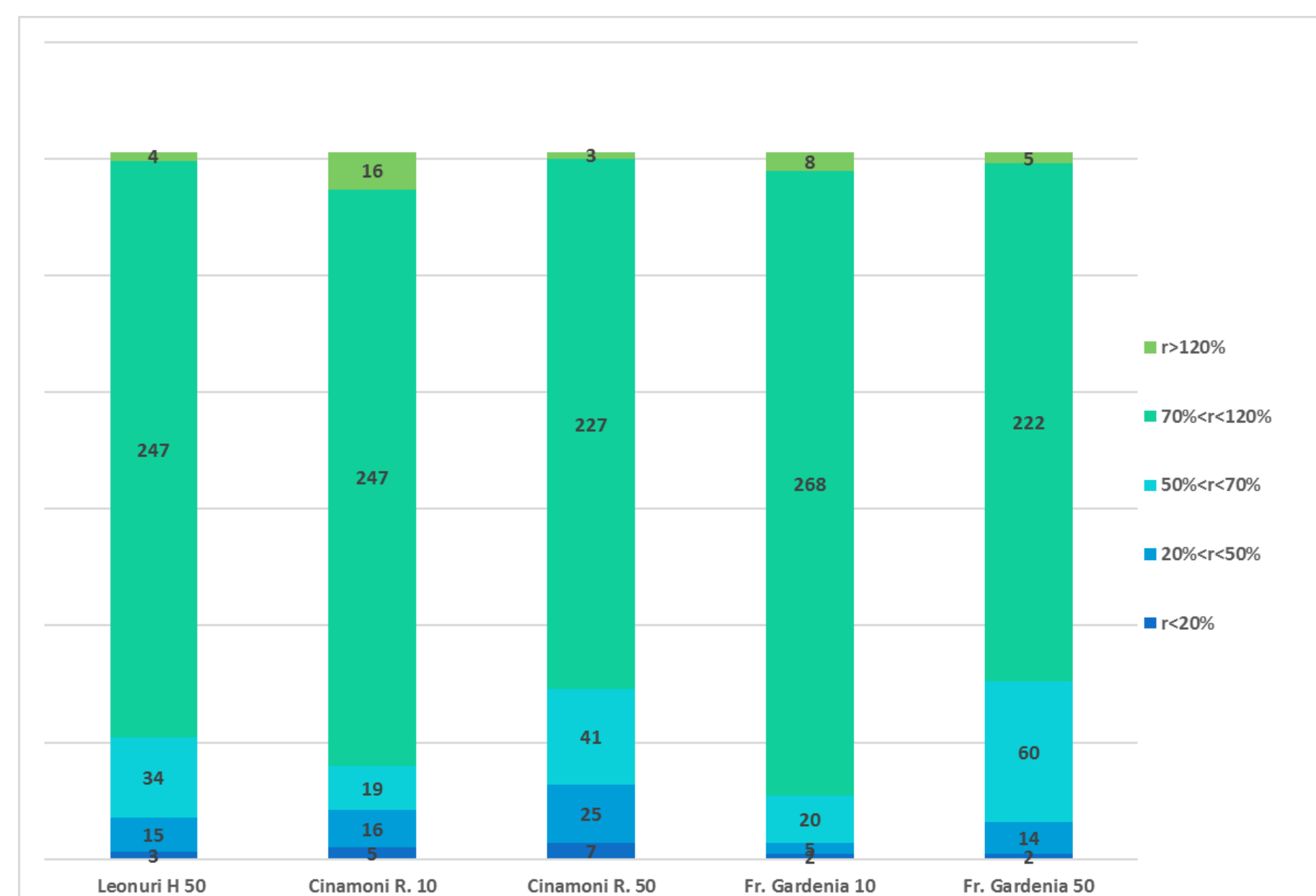


Figure 3. Extraction recoveries for 303 standard compounds in five different drug samples

Conclusions

- A new multi-residue UHPLC/MS/MS method for screening and estimated quantification of pesticides, mycotoxins and pyrrolizidine alkaloids has been applied to 10 different herbal drugs which are frequently used in TCM, see Table 1
- A screening and quantitation method for 303 common compounds has been developed
- QuEChERS extraction and EMR cleanup have been applied sequentially to meet the expectations
- 82% of the standard compounds showed recoveries >70% after sample preparation according to SANTE guidelines
- 7 different pesticides have been detected and quantified from the samples, no pyrrolizidine alkaloids nor mycotoxins were found
- 4 detected pesticides were quantitatively estimated above the EU Maximum Residue Levels
- Multi-residue screening for toxic contaminants will increase the safety of herbal drugs to ensure they meet safety requirements

Table 2. UHPLC and 6495 LC/MS method parameters.

Column	Agilent Poroshell 120 EC-C18 2.1 x 100 mm, 2.7 µm, 20°C
Injection volume	1 µL
Mobile phase	5 mM NH ₄ formate + 0.1% formic acid in water and methanol
Flow rate	0.6 mL/min
Gradient	10%B held for 0.5min, increased to 20% in 1.5min then to 100% in 14min and held for 2min, then back to 10% in 0.1min (Total cycle time 20min)
MS parameters	Positive and negative ESI with Agilent Jet Stream, dynamic MRM

Extracts were analyzed using an Agilent 1290 Infinity UHPLC system, coupled to a highly sensitive dual ion funnel Agilent 6495 triple quadrupole LC/MS system. Further method details are given in Table 2.

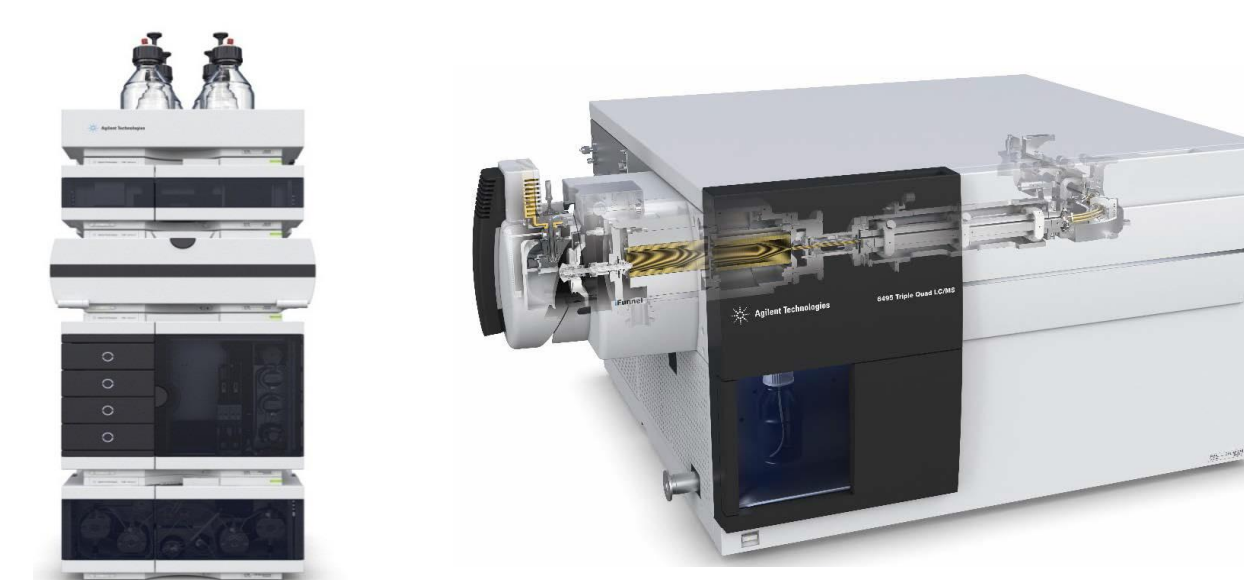


Figure 1. Agilent 1290 Infinity UHPLC - 6495 Triple Quadrupole MS

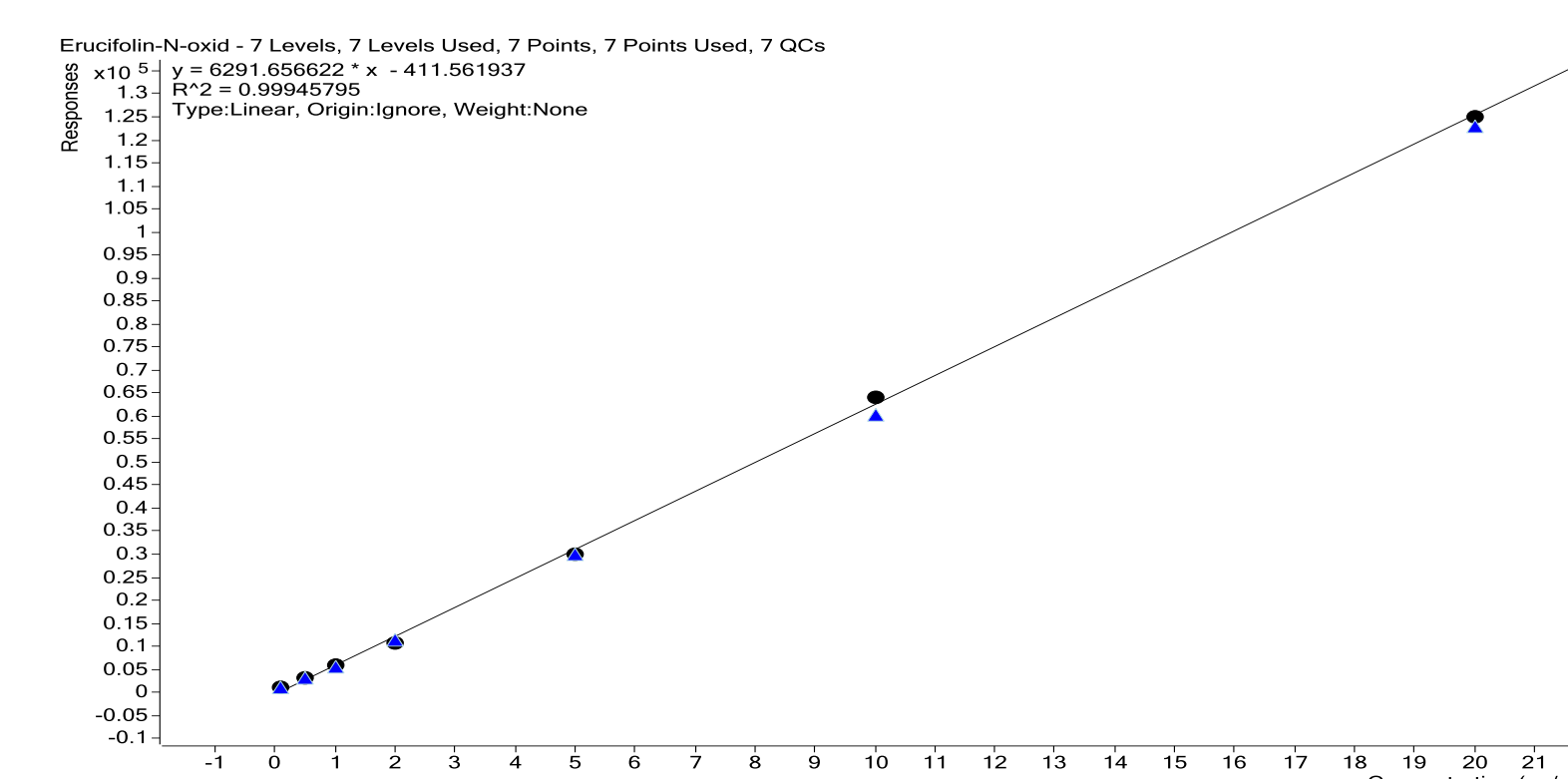


Figure 2. The described method delivered linear calibration curves of the standards in matrix. Example: Calibration curve for Erucifolin-N-oxid in matrix Mori folium

Analysis of TCM samples

The method was applied to the analysis of 10 different TCM samples purchased from a local pharmacy. Figure 4 shows the ion-chromatograms from detected pesticides in different TCM samples. The ion ratios of the qualifier ions are within the specifications.

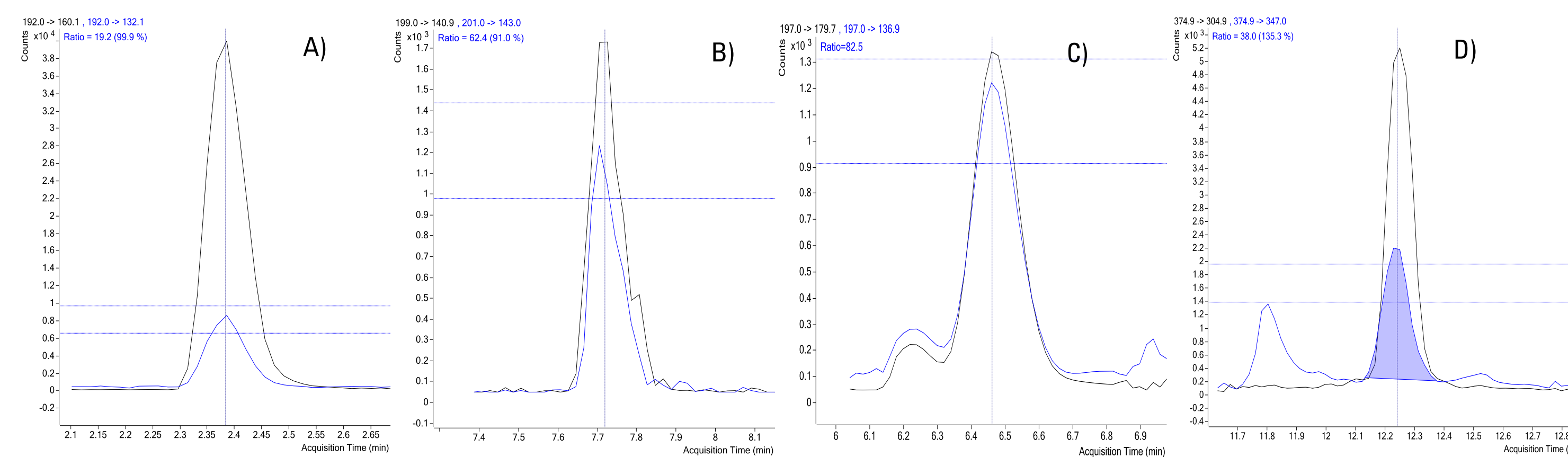


Figure 4. Pesticides detected in commercial TCM samples: (A) Carbendazim in Mori folium, (B) MCPA in Herba epimedii, (C) DNOC in Flos magnoliae, and (D) Profenofos in Cinamoni ramulus

In total residues of 7 different pesticides were found in the analyzed sample material. No mycotoxins nor pyrrolizidine alkaloids were found. The concentrations levels have been determined between 0.8 and 185.7 µg/kg. Three of the herbal drug samples contained at least one pesticide with an estimated concentration level superior to the European maximum residue limits (highlighted in red)^{ref 4}. The results are summarized in Table 4.

Table 4. Results: determined residue levels

Mori folium	Buprofezin (1.9ppb)	Carbendazim (185.7ppb)	Chlorpyrifos (62.1ppb)	DNOC (71.2ppb)
Herba epimedii	MCPA (116ppb)			
Leonuri herba	Carbendazim (63.5ppb)	DNOC (18.6ppb)		
Cinamoni ramulus	Buprofezin (1.3ppb)	Profenofos (6.1ppb)		
Radix ginseng	Carbendazim (8.6ppb)	Propargite (0.8ppb)		
Flos magnoliae	DNOC (165.5ppb)			

Discussion

Carbendazim has been detected in numerous samples. It has been shown that Carbendazim is not only a fungicide itself but also a degradation product of Thiophanat-methyl and Benomyl ^{ref 6}. Therefore, the presence of Carbendazim observed in this study may also be due to the degradation of other residues which might be present at higher concentration levels in two of the samples. DNOC is a banned compound in the EU since 1991 and has been found in Mori folium and Flos magnoliae samples. This focused and non-representative study from only 10 different TCM drugs, which were purchased in a pharmacy and are certificated for purity and authenticity, shows that there are safety gaps regarding the maximum residue levels for pesticides. These gaps could be closed with the use of a robust and precise UHPLC-MS/MS multi-residue screening process.

References:

1. Ferrer Amate, C. et al. (2010) Anal Bioanal Chem 397:93-107.
2. Bolechová, M. et al. (2015) Food Chemistry 170:265-270.
3. Sack, C. et al. (2015) J. Agric. Food Chem. 63:9657-9665.
4. EU pesticides database based on Regulation EC 396/2005
5. SANTE/11945/2015 guidelines for recoveries
6. Deutsche Lebensmittel-Rundschau, September 2013, 467-473