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# Determination of Pesticide Residues by GC-MS in Commercialized Mint Samples

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## Abstract

The sale of fresh mint in Morocco is not strictly controlled as for that which is intended for export to the European Union, which increases the risks of the contamination of mint tea consumers and constitutes a problem of public health. The method of dosing pesticides in fresh mint by GC-MS that we have developed and validated allowed us to high light contamination by pesticide residues (carbofuran, bifenthrin, chlopyriphos methyl and Fenpropathrin) in samples of mint fresh from sampled sale and temara, this contamination exceeds the MRLs. Regular control of the sale of fresh mint should be practiced to prevent repetitive contamination of local consumers by pesticide residues.

**Keywords:** Pesticides; Mint; Dosing; GC-MS

## Introduction

Spearmint or mint, *Mentha spicata* L cv. *viridis* [1] or *Mentha viridis* L [2], is a perennial of the family Limiaceae. In Morocco, spearmint is widely used with tea which is a national beverage and has been a medicinal plant for decades [3]. Like other vegetal, spearmint has phytosanitary problems due to pests (aphids, leafhoppers, moths, white flies, etc.) [4]. To control these bio-aggressors, which often generate significant losses, farmers treat their fields with synthetic pesticides; against fungal diseases, fungicides such as bitertanol, boscalid, triadimenol, etc., whereas against pests it is mainly treated with cypermethrin, permethrin and endosulfan [5,6]. In Morocco, no synthetic pesticides have been registered on mint cultivation [7,8]. However, if the use of pesticides has been considered for years as the most cost-effective means of control, the profit margin is considerably reduced by subtracting the costs of their undesirable effects on the environment and human health [9]. Pesticides all have varying degrees of potential for toxicity and can unfortunately be toxic to non-target organisms, including humans [10]. By their serious side effects on human health and the environment [11]; Consumers, users or those who are generally infected may face health risks such as suppression of the immune system, impairment of intellectual capacity, hormonal disruption, cancer, etc. [12] According to the World Health Organization, the annual number of pesticide poisonings is about 5 million, children, infants, and the elderly are the most sensitive. Male nutrition and dehydration increase susceptibility to pesticides [13]. It is the areas with high agricultural activity that are most exposed to the risks of pesticides [14]. In Morocco almost 7% of poisoning is of chemical origin, most of which is associated with the contamination of food by pesticides [15]. Exposure to pesticides can be through the respiratory, dermal and oral routes, the risk of which generally increases with the dose that does not necessarily improve the efficacy of the treatment [10]. Derived metabolites may also have a very disturbing toxicological profile. Their quantities and that of the parent molecule in or on the consumable parts of the plant at harvest depend on the nature of the pesticide, the climate, the plant being treated, the conditions of use, the formulation and the time to harvest (DAR) [16]. Moroccan fresh mint exported to the European Union (EU) is experiencing exceedances of the maximum residue limits (MRL) set by the latter [17,18], while several local sales outlets are not controlled by lack of effective methods of pesticide residues in Moroccan fresh mint, from which the primary objective of this study is to develop and validate a new method for the determination of pesticide residues in mint by GC-MS and to apply it to mint samples marketed locally.

## Materials and Methods

### Instrumentation

The gas chromatographic system coupled to mass spectrometry is PerkinElmer GC-MS Clarus 600/560DMS equipped with an automatic injector. The system is driven by a software Turbo Mass Software (Microsoft Windows XP SP2). The stationary phase is a Supelco® column (L 30 m × ID 0.25 X DF 0.25) of Elite-5MS phase, the carrier gas is helium at a flow rate of 0.8 mL/min. The oven was programmed from 90°C up to 230°C at a gradient of 15°C per min and then at 5°C per min up to 290°C with prior heating of the transfer line to 300°C and the ionization source at 250°C. The automatic injection is in splitless mode (50/1 to 250°C). Ionization is caused by an electronic impact (IE).

### Reagents and chemicals

Pesticide standards that have been used in the calibration range are LGC Standards.

- Acetonitrile, Toluene and Acetic acid.

- SPEXQuE QUECHERS KITS (Citrate Bufer Extraction Tubes).

- ULTRA QuECH (QUEC-208).

### Calibration range

Preparation of the calibration range (0.02 ppm, 0.05 ppm, 0.1 ppm, 0.2 ppm and 0.5 ppm) was made from the stock solution of a mixture of

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18 pesticides (Aminocarb, Ethoprophos, Carbofuran, Chlorpropham, Chlorbufam, Methyl Acibenzolar, Methyl Chlorpyriphos, Fenpropidin, Diethiofencarb, Cyprodinil, βendosulfan, Clodinafopropargyl, Buprofizin, Fenpropatrin, Fenarimol, Permetrin, Fenbuconazol and Boscalid) at 1 ppm by successive dilutions. The sulfotep was used as an internal standard at 0.1 ppm.

### Collection and origin of samples of mint

The samples of mint are brought from different sales outlets in the region of Rabat sale.

### Extraction by the method of QuECHERS

**Extraction:** Transfer 5-10 g of the mint sample homogenate into a 50 mL conical tube, add 10 mL of the acetinitrile solution containing 1% acetic acid, 100 µL of the internal standard and mix with the SPEXQuE Acetate Tube container (AOAC-ACE-50 mL). Vortex for 1 min and centrifuge at 3000 g for 5 min.

**Purification:** Transfer 6 ml of the organic phase into SPEXQuE PSA Tube (EN-PSA-15 mL) or 1 mL into SPEXQuE PSA Tube, 2 mL (ULTRA QuECH-QUEC-208). Vortex for 1 min and centrifuge at 3000 rpm for 5 min. Fill a glass tube with 1 mL of the extract and evaporate to dryness under a stream of nitrogen. Resume the dry extract with 100 µl of toluene and vortex for 1 min. Transfer the extract into the injection vial and inject 3 µl in splitless mode (Table 1).

## Results and Discussion

### Development of the method of dosage

After analysis, the chromatogram showing the peaks of the pesticides that make up the mixture was recorded in Figure 1 and the numerical results, Standard concentration (Cc), Retention time (TR), Concentration found (CT) and Yield (R) have been represented in Table 2. The electron impact molecular fragmentation of the mixing pesticides gave characteristic mass spectra for each product, some examples are shown in Figure 2. The most abundant mass peaks were selected for the development and validation of the assay method (Figure 3).

### Validation of the method of determination

The criteria to be evaluated during the validation of this analytical method are; selectivity, linearity, fidelity, limits of quantification (LOQ) and inter-sample contamination.

**Selectivity:** The blank extract from the extraction of a sample of organic mint showed no signal at the retention times of different pesticides in the mixture (Figure 4).

**Linearity:** Over a dosing interval consisting of 5 concentration levels (0.02 ppm, 0.05 ppm, 0.1 ppm, 0.2 ppm and 0.5 ppm), each concentration was repeated 3 times. The equation of the calibration line (Y=ax+b) for each pesticide was calculated by the least squares method across the range (Figure 5) with satisfactory R2 repeat coefficients (Table 2).

**Repeatability:** The repeatability of this method was studied at 0.1 ppm, this concentration was repeated 5 times. The coefficients of variation (CVr) calculated are less than 15% for all the pesticides in the mixture (Table 3).

**Reproducibility:** Reproducibility was performed on three independent series. The coefficients of variation (CVR) calculated are less than 15% for all the pesticides in the mixture (Table 3).

**Quantation limit (LOQ):** 5 samples of mint at 0.02 ppm of pesticides were analyzed under conditions of fidelity. The results gave satisfactory CV% coefficients of variation (Table 3).

**Inter sample contamination:** The injection of extracts of the organic mint just after the injection of mint extracts at 0.5 ppm under conditions of fidelity showed no inter-sample contamination for all the pesticides in the mixture. The CV% of the validation criteria are all less than 15% which confirms that this method is valid and we can apply it on mint samples that we have made (Table 1).

### Determination of pesticide residues in commercial mint samples

The application of the method of determination of pesticide residues in mint by GC-MS that we validated on mint samples marketed showed that among the 16 samples analyzed we found pesticide residues in 3 samples (MST4, MSJ11 and MTM14), see Figure 5 and Table 4. The persistence of pesticide residues (permethrin, carbufuran, chlorpyrifos methyl and fenpropathrin) in the mint samples marketed at Sale and Temara is only the result of non-compliance with the pre-harvest intervals (PHI) for each pesticide (Table 4) and misuse of these pesticides during treatment by failure to follow the instructions for each product [19].

## Conclusion

We validated a method for the determination of pesticide residues by GC-MS in mint. This method allowed us to evaluate the contamination of some samples of mint marketed locally. The results show the presence of pesticide residues in some samples at concentrations above the MRLs. This study will have to be extended to other regions to take the necessary measures to preserve the health of consumers.

| Mints Code | Origine | Amount | Nature |
|---|---|---|---|
| MST1 | Salé tabrékte | 115 g | Fresh mint |
| MST2 | Salé tabrékte | 103 g | Fresh mint |
| MST3 | Salé tabrékte | 111 g | Fresh mint |
| MST4 | Salé tabrékte | 110 g | Fresh mint |
| MST5 | Salé tabrékte | 102 g | Fresh mint |
| MSK6 | Salé hay karima | 113 g | Fresh mint |
| MSJ7 | Salé eljadida | 117 g | Fresh mint |
| MSJ8 | Salé eljadida | 111 g | Fresh mint |
| MSJ9 | Salé eljadida | 119 g | Fresh mint |
| MSJ10 | Salé eljadida | 122 g | Fresh mint |
| MSJ11 | Salé eljadida | 131 g | Fresh mint |
| MRA12 | Rabat agdal | 105 g | Fresh mint |
| MTG13 | Témara guiche loudaya | 110 g | Fresh mint |
| MTM14 | Témara elmassira | 111 g | Fresh mint |
| MTC15 | Témara centre | 120 g | Fresh mint |
| MTN16 | Témara hay enahda | 118 g | Fresh mint |

**Table 1:** Origins of mint samples.

| Pesticides | $C^c$ (ppm) | TR (min) | Masses pics (m/z) | Pics Qt (m/z) | CT (ppm) | R (%) |
|---|---|---|---|---|---|---|
| Carbofuran | 0.1 | 5.96 | 164,149,122 | 164 | 0.07 | 70 |
| Aminocarb | 0.1 | 6.34 | 151,150,136 | 151 | 0.06 | 60 |
| Ethoprophos | 0.1 | 8.59 | 158,43,97 | 158 | 0.08 | 80 |
| Chlorpropham | 0.1 | 8.77 | 127,43,213 | 127 | 0.07 | 70 |
| Chlorbufam | 0.1 | 9.45 | 53,127,223 | 53 | 0.07 | 70 |
| Chlorpyriphos méthyle | 0.1 | 10. 52 | 125,286,79 | 125 | 0.10 | 100 |
| Acibenzolar méthyle | 0.1 | 10. 65 | 63,182,107 | 63 | 0.09 | 90 |
| Fenpropidin | 0.1 | 10.78 | 98,99,55 | 98 | 0.08 | 80 |
| Diethiofencarb | 0.1 | 11.16 | 43,124,225 | 124 | 0.09 | 90 |
| Cyprodinil | 0.1 | 11.67 | 224,225,77 | 224 | 0.08 | 80 |
| β endosulfan | 0.1 | 12.61 | 207,170,69 | 207 | 0.06 | 60 |
| Clodinafopropargyl | 0.1 | 13.09 | 238,266,130 | 238 | 0.08 | 80 |
| Buprofizin | 0.1 | 13.13 | 105,106,57 | 105 | 0.09 | 90 |
| Fenpropatrin | 0.1 | 16.24 | 97,55,181 | 97 | 0.09 | 90 |
| Fenarimol | 0.1 | 17.87 | 139,107,219 | 139 | 0.08 | 80 |
| Permethrin | 0.1 | 18.76 | 183,91,77 | 183 | 0.08 | 80 |
| Fenbuconazol | 0.1 | 19.75 | 129,198,125 | 129 | 0.09 | 90 |
| Boscalid | 0.1 | 20.43 | 140,112,142 | 140 | 0.08 | 80 |
| **R Moy (%)** | | | | | | **75.78** |

**Table 2:** Analytical Parameters of Mixture Pesticides.

| Pesticides | TR (min) | R | LOQ (µg/ml) | CV% | CVr% | CVR% |
|---|---|---|---|---|---|---|
| Carbofuran | 5.96 | 0.996 | 0.02 | 5.70 | 3.21 | 4.99 |
| Aminocarb | 6.34 | 0.993 | 0.02 | 6.57 | 0.7 | 3.21 |
| Ethoprophos | 8.59 | 0.938 | 0.02 | 6.37 | 9.91 | 6.30 |
| Chlorpropham | 8.77 | 0.984 | 0.02 | 3.99 | 0.53 | 1.85 |
| Chlorbufam | 9.45 | 0.992 | 0.02 | 1.53 | 0.42 | 4.47 |
| Acibenzolar méthyle | 10.65 | 0.998 | 0.02 | 2.91 | 0.54 | 4.02 |
| Chlorpyriphos méthyle | 10.52 | 0.998 | 0.02 | 3.41 | 1.24 | 5.06 |
| Fenpropidin | 10.78 | 0.998 | 0.02 | 3.24 | 1.14 | 5.43 |
| Diethiofencarb | 11.16 | 0.999 | 0.02 | 3.04 | 0.92 | 4.64 |
| Cyprodinil | 11.67 | 0.998 | 0.02 | 4.08 | 1.63 | 4.23 |
| β endosulfan | 12.61 | 0.977 | 0.02 | 5.00 | 2.97 | 6.17 |
| Clodinafopropargyl | 13.09 | 0.998 | 0.02 | 4.98 | 2.97 | 4.56 |
| Buprofizin | 13.13 | 0.999 | 0.02 | 2.24 | 0.88 | 3.49 |
| Fenpropatrin | 16.25 | 0.999 | 0.02 | 2.99 | 0.43 | 5.02 |
| Fenarimol | 17.87 | 0.998 | 0.02 | 7.22 | 0.44 | 6.43 |
| Permethrin | 18.76 | 0.998 | 0.02 | 6.28 | 2.45 | 5.25 |
| Fenbuconazol | 19.75 | 0.995 | 0.02 | 4.13 | 3.84 | 6.17 |
| Boscalid | 20.43 | 0.998 | 0.02 | 4.95 | 2.55 | 5.29 |

**Table 3:** Results of Validation of the Method for Determination of Pesticide Residues in Mint by GC-MS.

| Mint Code | Pesticides detected (mg/kg) | LMR UE (mg/kg) | DAR (days) | Interpretation |
|---|---|---|---|---|
| MST1 | Negative | - | - | - |
| MST2 | Negative | - | - | - |
| MST3 | Negative | - | - | - |
| MST4 | Permithrin (0.35) | 0.2 | 25 | >LMR UE |
| MST5 | Negative | - | - | - |
| MSK6 | Negative | - | - | - |
| MSJ7 | Negative | - | - | - |
| MSJ8 | Negative | - | - | - |
| MSJ9 | Negative | - | - | - |
| MSJ10 | Negative | - | - | - |
| MSJ11 | Carbofuran (0.28) | ND | ND | - |
| MRA12 | Negative | - | - | - |
| MTG13 | Negative | - | - | - |
| MTM14 | Chlorpyriphos méthyle (0.12) | 0.05 | 30 | > LMR UE |
| MTM14 | Fenpropathrin (0.11) | ND | ND | - |
| MTC15 | Negative | - | - | - |
| MTN16 | Negative | - | - | - |

**Table 4:** Results of Pesticide Residues in Mint Samples by GC-MS.

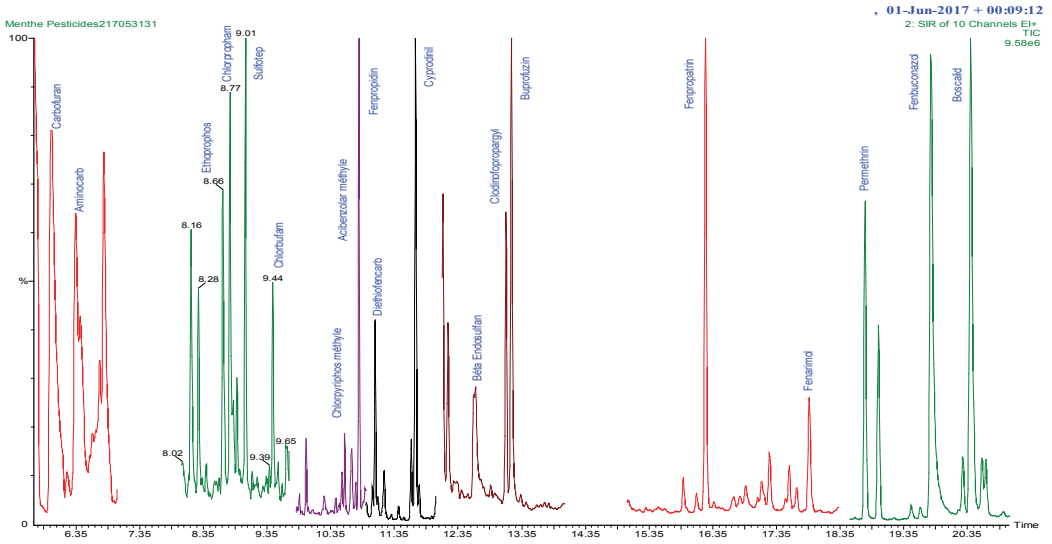


**Figure 1:** Chromatogram of pesticides in the calibration range.

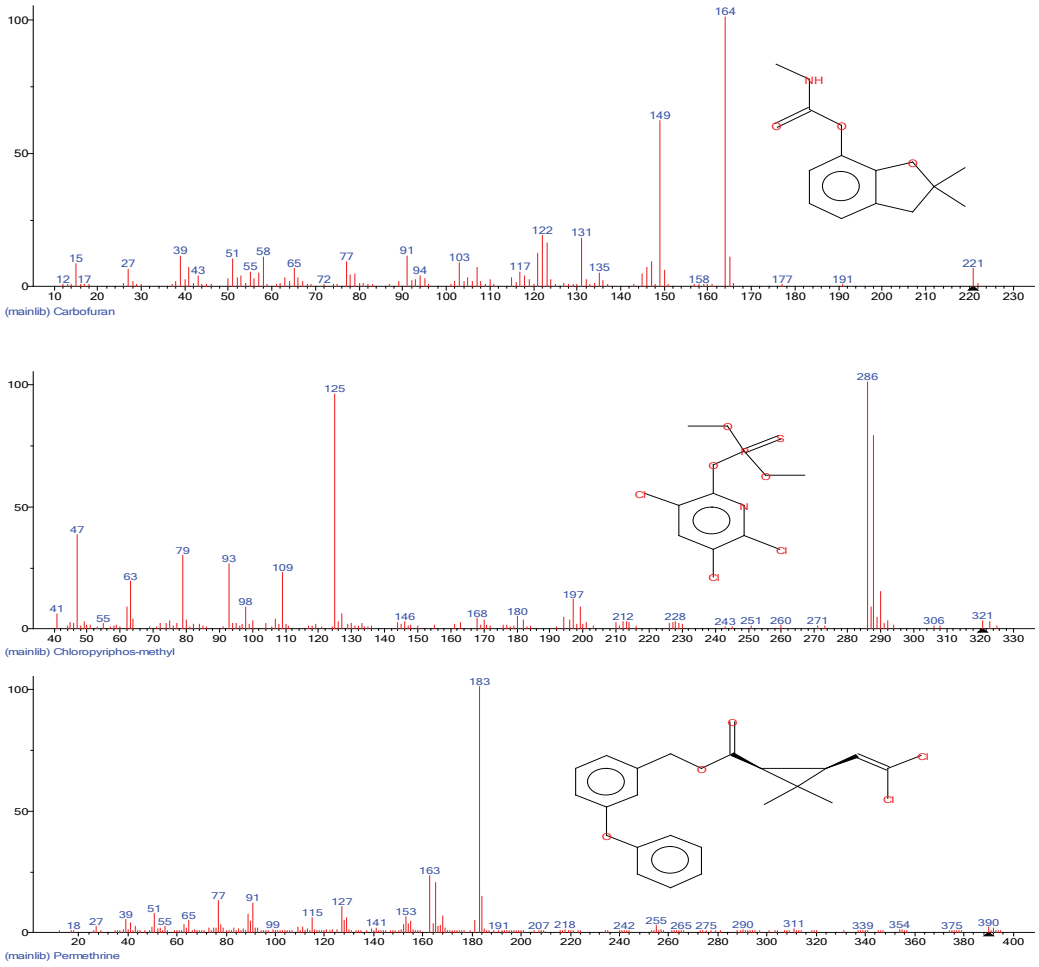


**Figure 2:** Example of mass spectra of pesticides in the calibration range.

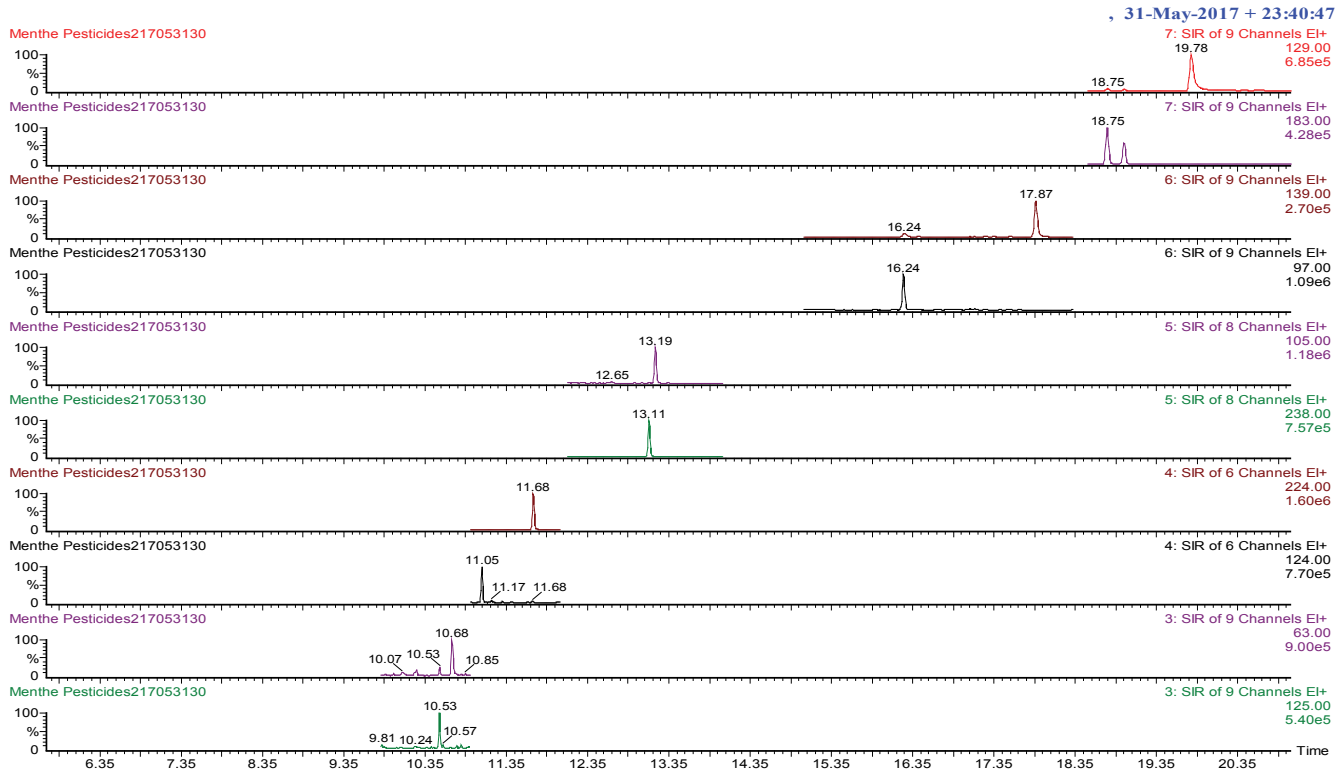


**Figure 3:** Example of choice of mass peaks for the dosing method.

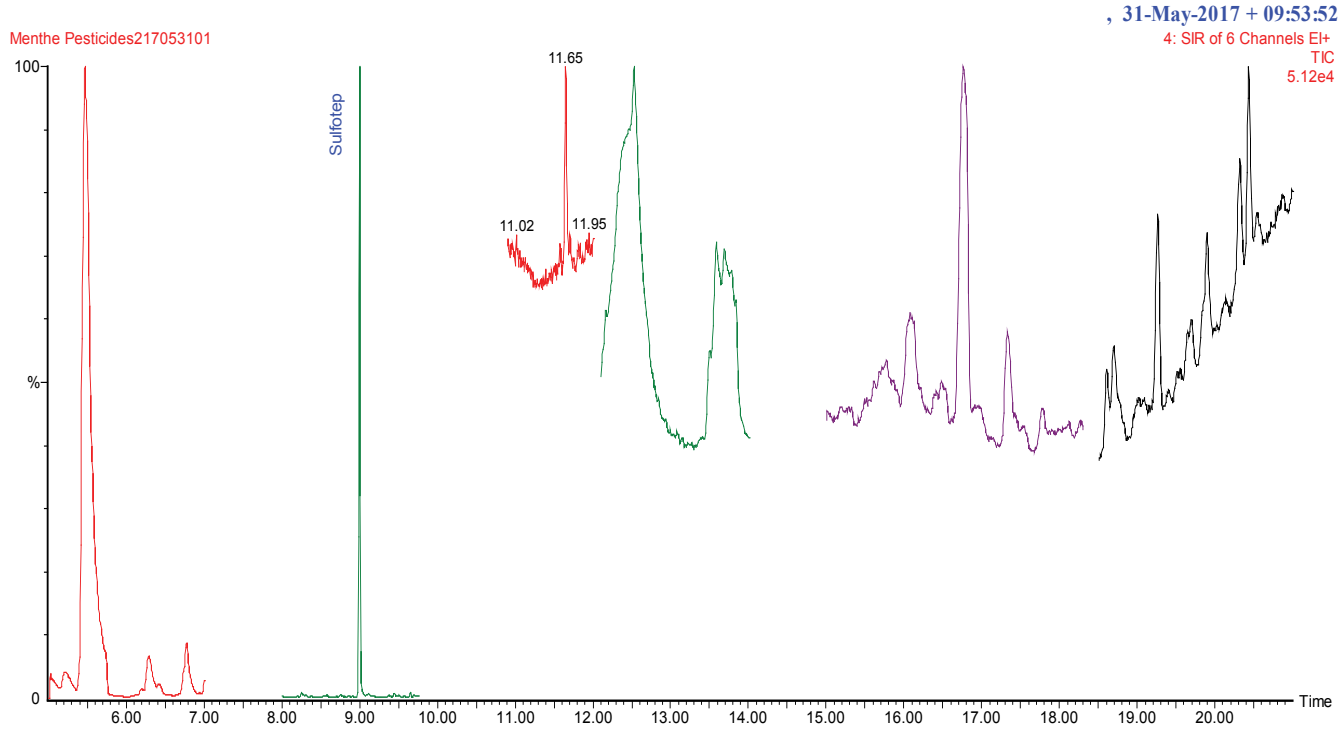


**Figure 4:** Chromatogram of selectivity.

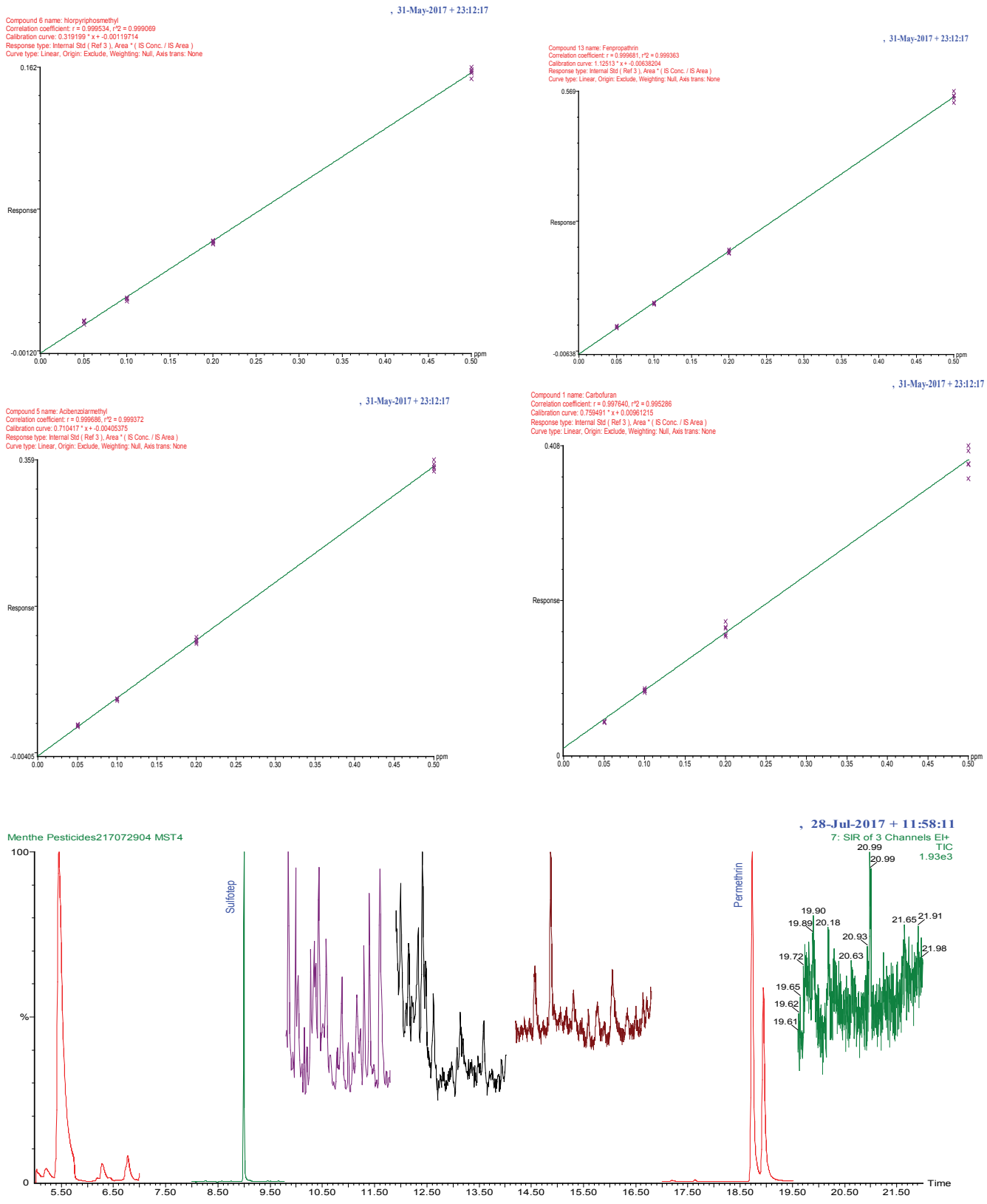


**Figure 5:** Chromatogram of a mint extract (MST4) contaminated with permethrin.

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