M. Biendl, G. Jaeger, R. Kittsteiner-Eberle and C. Schmidt

Rapid and Sensitive Determination of Pesticide Residues in Hops and Hop Products Using HPLC-MS/MS and GC-MS/MS

A rapid method was developed to determine the residue levels of 46 pesticides in unprocessed hops and hop products (pellets, extract). This method utilizes liquid extraction, solid phase extraction, and finally gas chromatography or liquid chromatography, each combined with tandem mass spectrometry. For hops and hop pellets, a limit of quantification of 0.1 mg/kg or lower could be achieved for all active compounds. The limits of quantification are always a factor of two higher for hop extract.

Descriptors: pesticides, multi-residue method, LC-MS/MS, GC-MS/MS, hops, hop pellets, hop extract

1 Introduction

Hop growers strive to achieve careful balance between combating pests or pathogens and producing a crop that can pass the import tolerances or maximum residue levels (MRLs) imposed by the various countries to which hops are exported.

In order to monitor such MRLs, sophisticated analysis methods for measuring residues in hops or hop products were introduced in the German hop industry since more than 20 years ago [1].

Unfortunately, universal standard methods for analysing different crops are not very applicable to hops due to the more complex matrix of this plant known to be high in the content of low molecular weight bitter acids. One of the first modified residue analysis methods suitable for hops was published by *Fuchsbichler* and *Tkaczyk* in 1989 [2]. They suggested an alternative gel permeation chromatography (GPC) procedure as compared to the "DFG S-19" ("Specht") method [3], which was the most recognized residue method in Germany at that time and which still serves as an official standard method [4].

Until ten years ago, routine pesticide residue analysis was mainly conducted using gas chromatography (GC) in combination with electron capture, nitrogen-phosphorous, and/or flame photometric detection (ECD, NPD, FPD). Confirmation of results usually required the use of a second gas chromatograph equipped with a different type of column or detector. Nowadays, using GC combined with mass spectrometry (MS), simultaneous determination and confirmation of pesticide residues can be obtained with a single instrument in one analytical run. In most cases, the sensitivity obtained with GC-MS is similar to that of classic GC detectors. Therefore, the importance of GC with ECD, NPD or FPD detection has decreased in laboratories testing for pesticide residues. The selectivity of MS detection is further improved by the so-called tandem mass spectrometric (MS/MS) detection technique involving two stages of mass analysis.

Today, tandem mass spectrometry coupled with gas chromatography (GC-MS/MS) or with liquid chromatography (LC-MS/MS) is considered state-of-the-art in modern analysis for complete characterization of pesticides and their full range of functional groups. A review presenting these techniques was published by *Alder et al.* [5].

Since selective tandem mass spectrometry has become established even for routine pesticide analysis, more and more laboratories are moving away from time-consuming gel permeation chromatography when analysing pesticide residues. Such methods abbreviated as "QuEChERS" (Quick, Easy, Cheap, Effective, Rugged and Safe) are widely established and officially recommended [6–9]. But again, due to the high resin content, these methods are hardly applicable to hops without further modification. A suitable multi-residue method for hops which does not involve gel permeation chromatography was published by *Hengel* [10]. This modified "QuEChERS" method utilizes acetonitrile extraction, solid-phase extraction (SPE), and liquid chromatography with tandem mass spectrometry (LC-MS/MS). It is used to determine the residue levels of 28 pesticides in dried hop cones. For screening a broad range of totally 310 pesticides in hops or other botanicals a similar sample preparation followed by gas chromatography with tandem mass spectrometry (GC-MS/MS) was described as applicable [11].

In the current study, another sample preparation method was developed without incorporating the time-consuming gel permeation chromatography technique for the reliable determination of pesticide residues in dried hops or in hop products like pellets and extracts. After liquid extraction, the following sample purification steps are split into two procedures, one being suitable for LC-MS/MS analysis and the other for GC-MS/MS analysis. In both cases, only solid phase extraction is applied.

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This multi-residue method can be utilized for screening purposes immediately after harvest as well as for quality control throughout processing.

2 Materials and methods

2.1 Reagents

Following chemicals were obtained from commercial sources: water and methanol for LC-MS, acetone, toluene, hexane and ethyl acetate for pesticide residue analysis (Chemsolute®, Th. Geyer GmbH & Co. KG, Renningen, Germany); cyclohexane, dichloromethane and acetonitrile for pesticide residue analysis (Prolabo®, VWR, Darmstadt, Germany); sodium chloride, formic acid and ammonium formate (Sigma Aldrich, Steinheim, Germany).

Pesticide standards acetamiprid, abamectin, azoxystrobin, bifenthrin, boscalid, captan, carbofuran, cinidon-ethyl, chlorantraniliprole, clothianidin, cymoxanil, cypermethrin, diazinon, dimethomorph, etoxazole, fenpropimorph, fenpyroximate, fipronil, flonicamid, fluazifop-p-butyl, fluopicolide, folpet, hexythiazox, imidacloprid, lambda-cyhalotrin, linuron, mandipropamid, metalaxyl, methidathion, myclobutanil, propamocarb, propargite, pymetrozine, pyraclostrobin, pyraflufen-ethyl, quinoxyfen, spirodiclofen, spiroxamine, tebuconazole, terbuthylazine, thiacloprid, thiamethoxam, tolylfluanid, triadimenol, trifloxystrobin, and zoxamide were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Internal standards folpet D4, captan D6, PCB No. 138, PCB No. 174, and PCB No. 209 (for GC analysis) as well as dimefuron and dimetilan (for LC analysis) were also sourced from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

The following materials were also obtained commercially: magnesium silicate (Florisil® for TLC, Sigma Aldrich, Steinheim, Germany), silica gel 60 (Merck KGaA, Darmstadt, Germany), and Mega Bond Elut-PSA cartridges (1 g/6 ml, Agilent Technologies, Boeblingen, Germany).

2.2 Sample preparation for unprocessed (dried) hop cones, hop pellets and hop extract for GC-MS/MS analysis

5 g of milled hop cones or pellets were weighed into a 250 ml screw cap bottle, and 50 ml of water and 100 ml of acetone were added. The sample was extracted using a shaking device (225 rpm) for 30 min. An aliquot (30 mL) of the supernatant was removed, mixed with 6 ml of a saturated sodium chloride solution and 10 ml of dichloromethane and then shaken. To achieve phase separation, the sample was centrifuged for 2 min at 2000 rpm. An aliquot of the organic phase (7.5 ml) was evaporated until dryness, suspended in 10 ml of cyclohexane/dichloromethane mixture (85/15, v/v), dissolved using an ultrasonic bath and transferred quantitatively to a disposable 50 ml centrifuge tube. The sample was centrifuged at 2000 rpm for 2 min.

0.5 g of the hop extract (ethanol or carbon dioxide extract) was weighed into a centrifuge tube and 50 ml of cyclohexane/dichloromethane mixture (85/15, v/v) was added. The sample was extracted with help of an ultrasonic bath (10 min), then centrifuged for 2 min at 2000 rpm and filtered. The resulting solution was evaporated until dryness, suspended in 10 ml of cyclohexane/dichloromethane mixture (85/15, v/v), dissolved using an ultrasonic bath and transferred quantitatively to a disposable 50 ml centrifuge tube. The sample was centrifuged at 2000 rpm for 2 min.

For solid phase extraction (SPE), a cartridge packed with silica gel (4 g of silica gel 60) was preconditioned with 10 ml of hexane and another cartridge filled with magnesium silicate (5 g of Florisil®) was preconditioned with toluene (10 ml). Both cartridges were then interconnected (silica gel above magnesium silicate).

2.5 ml (for hop cones and pellets) or 0.5 ml (for hop extract) of the sample extract solution obtained was transferred to the connected cartridges which were eluted in sequence with 30 ml of toluene/acetone mixture (95/5, v/v) into a TurboVap tube. The resulting solution was then evaporated until dryness using a TurboVap workstation (Caliper Life Sciences, Hopkinton, USA) and dissolved in 0.5 ml of toluene. Mixture of internal standards for GC analysis (final concentration: 100 ng/ml) was added.

2.3 Sample preparation for unprocessed (dried) hop cones, hop pellets and hop extract for LC-MS/MS analysis

5 g of milled hop cones or pellets were weighed into a 250 ml screw cap bottle and 50 ml of water and 100 ml of acetone were added. The sample was extracted using a shaking device (225 rpm) for 30 min. To an aliquot (30 ml) of the supernatant 6 ml of a saturated sodium chloride solution and 10 mL of dichloromethane were added and the mixture was shaken. To achieve phase separation, the sample was centrifuged for 2 min at 2000 rpm. An aliquot of the organic phase (7.5 ml) was evaporated until dryness, suspended in 10 ml of ethyl acetate, dissolved using an ultrasonic bath and transferred quantitatively to a disposable 50 ml centrifuge tube. The sample was centrifuged at 2000 rpm for 2 min.

0.5 g of the hop extract (ethanol or carbon dioxide extract) was weighed into a centrifuge tube and 50 ml of ethyl acetate was added. The sample was extracted with help of an ultrasonic bath (10 min), centrifuged for 2 min at 2000 rpm and filtered. The resulting solution was evaporated until dryness, suspended in 10 ml of ethyl acetate, dissolved using an ultrasonic bath and transferred quantitatively to a disposable 50 ml centrifuge tube. The sample was centrifuged at 2000 rpm for 2 min.

For SPE, a Mega Bond Elut-PSA (Primary Secondary Amine) cartridge was preconditioned with 10 ml of acetonitrile.

5 ml (for hop cones and pellets) or 1 ml (for hop extract) of the resulting sample extract solution were transferred to a PSA cartridge and eluted with 10 ml of acetonitrile into a TurboVap tube. The sample was evaporated until dryness using a TurboVap workstation (Caliper Life Sciences, Hopkinton, USA) and dissolved in 1 ml of LC-MS/MS eluent (A/B, 50/50, v/v). After the addition of the internal standard mixture for LC-MS/MS analysis (final concentration: 100 ng/ml), the sample was transferred to an Eppendorf safe-lock tube and centrifuged for 15 min at 13500 rpm. The supernatant

was filtered through a 0.45 µm syringe filter (PLEOMAX, Berrytec GmbH, Gruenwald, Germany) and analysed directly (or after dilution) via LC-MS/MS.

2.4 Sample analysis

2.4.1 Gas chromatography – tandem mass spectrometry (GC-MS/MS)

Sample measurements were carried out using a TRACE 1300 gas chromatograph with a temperature-programmed PTV injection system and an AS 3000 autosampler coupled with a TSQ 8000 Triple Quadrupole Mass Spectrometer (Thermo Scientific, Dreieich, Germany). Xcalibur and TraceFinder softwares (Thermo Scientific, Dreieich, Germany) were used for instrument control, analysis and data evaluation.

The GC-MS/MS conditions were as follows:

Injection volume	2 µl
Injector PTV	Splitless mode
Base temperature	90 °C
Transfer	14.5 °C/sec to 250 °C, for 3 min
Clean	14.5 °C/sec to 265 °C, for 1 min
Flow	Constant flow, 1.2 ml/min, helium
Analytical column	TG-5SILMS, 15 m, I.D. 0.25 mm, 0.25 µm film thickness
Column oven	Temperature-programmed
Start	95 °C, for 1 min
Ramp 1	30 °C/min to 150 °C
Ramp 2	20 °C/min to 265 °C, for 2 min
Ramp 3	50 °C/min to 320 °C, for 2 min
Scan type	Timed selected reaction monitoring (SRM) mode
Ionization	EI (positive)
MS transfer line temperature	280 °C
Ion source temperature	280 °C

The compound-specific parameters were optimized by means of automated SRM and are given in table 1.

Table 1 Compound-specific parameters for the GC-MS/MS analysis

Compound	Precursor mass (*m/z*)	Product mass (*m/z*)	CE[a] [V]
Bifenthrin	166.3	165.1	10.0
	181.1[b]	165.1[b]	24.0
Captan	117.0	82.0	28.0
	149.0[b]	70.0[b]	18.0
Captan D6 (IS)	154.1	70.0	14.0
	154.1[b]	84.0[b]	14.0
Cypermethrin	163.0[b]	127.1[b]	8.0
	164.9	127.1	8.0
Etoxazole	300.2[b]	270.2[b]	22.0
	300.2	285.2	12.0
Fipronil	367.0	213.0	28.0
	368.8[b]	215.0[b]	26.0
Folpet	259.9[b]	130.0[b]	15.0
	261.9	130.0	15.0
Folpet D4 (IS)	264.0[b]	134.1[b]	14.0
	265.8	134.1	14.0
Lambda–cyhalothrin	197.1	141.1	10.0
	208.0[b]	181.0[b]	8.0
Quinoxyfen	237.1[b]	208.1[b]	26.0
	307.1	237.1	18.0
PCB No. 138 (IS)	357.8	287.9	24.0
	359.9[b]	289.9[b]	26.0
PCB No. 174 (IS)	393.8[b]	323.9[b]	28.0
	393.8	359.0	10.0
PCB No. 209 (IS)	495.7	425.7	25.0
	497.7[b]	427.7[b]	25.0
Spirodiclofen	312.1	109.1	16.0
	312.1[b]	259.1[b]	10.0
Trifloxystrobin	116.0[b]	89.1[b]	14.0
	206.0	131.0	12.0

[a] Collision energy. [b] Quantifier ion. IS: Internal Standard

2.4.2 Liquid chromatography – tandem mass spectrometry (LC-MS/MS)

The HPLC system, consisting of a binary pump, a degasser, an autosampler and a thermostatted column oven (Shimadzu Corporation, Kyoto, Japan), was coupled with a 3200 Q-TRAP mass spectrometer (AB SCIEX, Darmstadt, Germany) equipped with the electrospray ionization (ESI) source running in the positive ion mode. Samples were introduced by HPLC at a solvent flow of 500 µl/min, which required the use of turbo gas at a temperature of 480 °C. The ion spray voltage was set to 5500 V, the declustering potential and the MS/MS parameters were optimized for each substance to induce fragmentation of the pseudo molecular ion $[M-H]^+$ to the corresponding target product ions after collision-induced

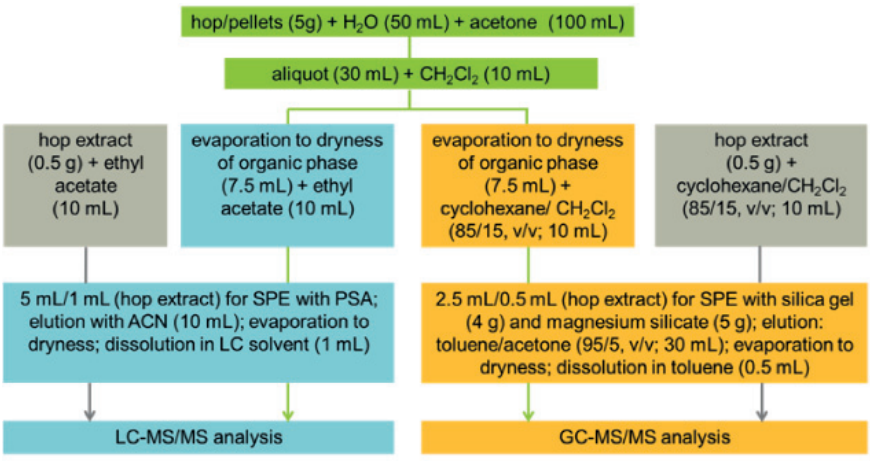

Fig. 1 Sample preparation scheme for unprocessed (dried) hops, hop pellets and hop extract

Table 2 Specific mass transitions and optimized parameters for the LC–MS/MS analysis of several active compounds

Compound	Mass transitions m/z Q1→Q3	DP[a] [V]	CE[b] [V]	CEP[c] [V]
Acetamiprid	223.1 → 126.0[d]	36.0	29.0	18.0
	223.1 → 73.1	36.0	71.0	18.0
Abamectin[e]	890.7 → 305.2[d]	21.5	35.0	35.0
	890.7 → 145.7	21.5	40.0	35.0
Azoxystrobin	404.1 → 372.2[d]	36.0	21.0	36.0
	404.1 → 329.2	36.0	37.0	36.0
Boscalid	343.1 → 307.2[d]	56.0	25.0	20.0
	343.1 → 140.1	56.0	27.0	20.0
Carbofuran	222.2 → 123.2[d]	36.0	29.0	20.0
	222.2 → 165.2	36.0	15.0	20.0
Chlorantraniliprole	484.0 → 286.0[d]	31.0	23.0	28.0
	484.0 → 453.0	31.0	23.0	28.0
Cinidon-ethyl	394.1 → 348.1[d]	36.0	21.0	30.0
	394.1 → 107.1	36.0	41.0	30.0
Clothianidin	250.0 → 132.0[d]	31.0	21.0	18.0
	250.0 → 169.2	31.0	17.0	18.0
Cymoxanil	199.1 → 128.1[d]	16.0	13.0	18.0
	199.1 → 111.2	16.0	23.0	18.0
Diazinon	305.2 → 169.2[d]	41.0	31.0	20.0
	305.2 → 153.2	41.0	31.0	20.0
Dimefuron (IS)	339.2 → 72.2[d]	56.0	47.0	20.0
	339.2 → 167.2	56.0	27.0	20.0
Dimethomorph[f]	388.2 → 301.2[d]	46.0	25.0	22.0
	388.2 → 165.3	46.0	41.0	22.0
Dimetilan (IS)	242.3 → 72.0	26.0	23.0	20.0
	242.3 → 73.0d	26.0	27.0	20.0
Etoxazole	359.9 → 141.2[d]	51.0	45.0	20.0
	359.9 → 113.1	51.0	81.0	20.0
Fenpropimorph	304.3 → 147.1[d]	61.0	39.0	20.0
	304.3 → 117.1	61.0	71.0	20.0
Fenpyroximate	422.3 → 366.2[d]	46.0	23.0	36.0
	422.3 → 107.2	46.0	79.0	36.0
Fipronil	437.0 → 368.2	61.0	23.0	28.0
	437.0 → 255.1[d]	61.0	43.0	28.0
Flonicamid	230.1 → 203.2[d]	46.0	21.0	18.0
	230.1 → 148.1	46.0	37.0	18.0
Fluazifop-p-butyl	384.2 → 282.2[d]	36.0	25.0	24.0
	384.2 → 328.2	36.0	21.0	24.0
Fluopicolide	383.1 → 173.1[d]	41.0	31.0	24.0
	383.1 → 109.1	41.0	89.0	24.0
Hexythiazox	353.2 → 228.1[d]	31.0	19.0	20.0
	353.2 → 168.2	31.0	31.0	20.0
Imidacloprid	256.1 → 209.2[d]	36.0	23.0	18.0
	256.1 → 175.1	36.0	25.0	18.0
Linuron	249.1 → 160.1[d]	36.0	23.0	18.0
	249.1 → 182.1	36.0	21.0	18.0
Mandipropamid	412.2 → 328.3[d]	41.0	19.0	36.0
	412.2 → 125.1	41.0	49.0	36.0
Metalaxyl	280.2 → 220.2[d]	21.0	17.0	18.0
	280.2 → 192.2	21.0	21.0	18.0
Methidathion	303.1 → 145.0[d]	26.0	15.0	18.0
	303.1 → 144.4	26.0	15.0	18.0
Myclobutanil	289.3 → 70.1[d]	46.0	33.0	18.0
	289.3 → 125.2	46.0	45.0	18.0
Propamocarb	189.2 → 102.2[d]	31.0	23.0	20.0
	189.2 → 74.1	31.0	35.0	20.0
Propargite	368.2 → 231.4[d]	26.0	15.0	22.0
	368.2 → 175.2	26.0	21.0	22.0
Pymetrozine	218.1 → 105.1[d]	41.0	27.0	18.0
	218.1 → 78.1	41.0	57.0	18.0
Pyraclostrobin	388.2 → 163.2[d]	26.0	31.0	22.0
	388.2 → 194.1	26.0	19.0	22.0
Pyraflufen-ethyl	413.0 → 339.1[d]	41.0	23.0	36.0
	413.0 → 253.1	41.0	45.0	36.0
Quinoxyfen	307.9 → 197.1[d]	66.0	45.0	18.0
	307.9 → 162.1	66.0	59.0	18.0
Spirodiclofen	411.2 → 71.1	41.0	27.0	28.0
	411.2 → 313.2[d]	41.0	19.0	28.8
Spiroxamine	298.4 → 144.2[d]	41.0	27.0	18.0
	298.4 → 100.2	41.0	43.0	18.0
Tebuconazole	308.2 → 70.1[d]	46.0	45.0	18.0
	308.2 → 125.1	46.0	53.0	18.0
Terbuthylazine	230.3 → 174.2[d]	36.0	23.0	20.0
	230.3 → 104.1	36.0	43.0	20.0
Thiacloprid	253.1 → 126.2[d]	46.0	29.0	20.0
	253.1 → 186.1	46.0	17.0	20.0
Thiamethoxam	292.1 → 211.2[d]	26.0	17.0	18.0
	292.1 → 181.2	26.0	27.0	18.0
Tolylfluanid	347.1 → 137.2[d]	36.0	35.0	20.0
	347.1 → 238.0	36.0	17.0	20.0
Triadimenol	296.1 → 70.1[d]	21.0	25.0	18.0
	296.1 → 99.1	21.0	19.0	18.0
Trifloxystrobin	409.0 → 186.2[d]	31.0	23.0	28.0
	409.0 → 145.2	31.0	65.0	28.0
Zoxamide	336.1 → 187.1	46.0	29.0	18.0
	336.1 → 159.1[d]	46.0	57.0	18.0

[a] Declustering potential
[b] Collision energy
[c] Cell entrance potential
[d] Quantifier ion
[e] Measured as avermectin B_{1a} (Abamectin, a mixture of 91.5 % avermectin B_{1a} and 3.5 % avermectin B_{1b})
[f] Sum of E and Z isomers. IS: Internal Standard

dissociation. The collision energy (CE), the declustering potential (DP) as well as the cell entrance potential (CEP) were set as given in table 2. Nitrogen was used as the collision gas. The quantitation was done using the scheduled multiple reaction monitoring (MRM) mode of the instrument with the fragmentation parameters optimized prior to analysis. Data processing and integration was performed by using Analyst software version 1.5.2 (AB SCIEX, Darmstadt, Germany). For chromatography, an analytical 50 x 2.0 mm Synergi 4µm Fusion-RP 80 Å column (Phenomenex, Aschaffenburg, Germany) equipped with a guard column of the same type (Phenomenex, Aschaffenburg, Germany) served as the stationary phase. 5 mM ammonium formate containing 0.1 % formic acid in water was used as solvent A and methanol with 5 mM ammonium formate and 0.1 % formic acid as solvent B. The temperature of the column oven was set at 40 °C. The injection volume was 10 µl. Chromatography was performed by increasing solvent B from 20 to 100 % within 8 min and holding for 2 min. Quantitation was done by external calibration in a range between 1 and 500 ng/ml.

3 Results and discussion

Figure 1 gives a schematic overview of the multi-residue method described above. Tables 1 and 2 present the MS conditions for the total of 46 active ingredients covered by our multi-residue method. Of these, 10 are analysed by GC-MS/MS. Some of them could also be measured with LC-MS/MS but in the end the limits of quantification and/or recovery rates were superior when using the GC-MS/MS route. For 36 compounds, LC-MS/MS analysis proved to be more efficient.

Table 3 compares the limits of quantification (LOQs) for all 46 compounds with the actual maximum residue levels (MRLs) as specified in different countries [12–14]. The LOQs do not exceed 0.1 mg/kg which is more than sufficient for almost all active compounds tested. However, if the EU MRL is below 0.1 mg/kg the LOQ was adjusted accordingly (see fluopicolide or captan for example). Only for fipronil, the very low MRL of 0.01 mg/kg required by the EU is not achieved. Although we are not able to precisely quantify such a low concentration using our multi-residue method, it can still be detected.

As is the case with hop extract, the bitter acids are heavily enriched and such samples are more difficult to purify. Therefore, all LOQs for hop extract are generally a factor of two higher as compared to hop cones or pellets. However, these LOQs are acceptable for extracts due to the so-called processing factor (see below).

Recovery rates achieved with our multi-residue method are presented in table 3, too. These investigations were carried out by adding 0.005 mg of a specific compound (in solution) to exactly 5 g of milled hop pellets free of any pesticide residues. Such pellets (resulting from organically grown hops) had been pre-selected prior to the experiment. All recovery rates were calculated by dividing the detected concentrations to the added amount of 1 ppm (multiplication by 100 yields the values in %). With the exception of propamocarb (22 %), pymetrozine (42 %) and spiroxamine (169 %), all rates were in the range of 60–130 %. In practice we determine the recovery rates for each compound several times a year at levels between 0.02–1 mg/kg depending on the specific MRL of an active ingredient. Usually the corresponding averages are between 70–120 %. In such cases, the recovery rates are not considered when calculating the analytical values resulting from our multi-residue method. Only for propamocarb, pymetrozine and spiroxamine must the actual recovery rates of the latest spiking experiments be always considered when expressing the results.

Since crop 2012, all batches of hop cones, hop pellets or hop extracts sold by the German branch of the international Hopsteiner group were carefully monitored using the multi-residue analysis method described above. All testing was carried out in the laboratory of the processing company.

In total, 115 hop cone samples, 342 pellet samples (both pellet type 90 and type 45) and 145 extract samples (both ethanol and carbon dioxide extract) were analysed during the processing season 2012/2013. Depending on the batch size, one sample represented a maximum of 30 tons. Of the entire list of 46 compounds, only 13 could be detected in the 602 samples. For the most part, there were residues of azoxystrobin, boscalid, dimethomorph, myclobutanil, mandipropamid or pyraclostrobin, whereas folpet, flonicamid, hexythiazox, quinoxyfen, spirodiclofen, triadimenol or trifloxystrobin could only be detected in very few samples. In the end, no result exhibited any regulated tolerance level and thus all products could be placed on the market. Considering the fact that only a few g of hops are added per litre during the brewing process, any residues coming from this raw material are harmless to consumers. Recently Kippenberger et al. reported that even hop pellets with unusually high contents of some pesticides are not a matter of concern for beer [15]. In this worst case study both conventional and dry hopping were considered.

Some pesticides are reduced to a certain degree during hop extraction. This means they are not fully transferred to the final extract. The order of magnitude for this reduction is dependent on both the

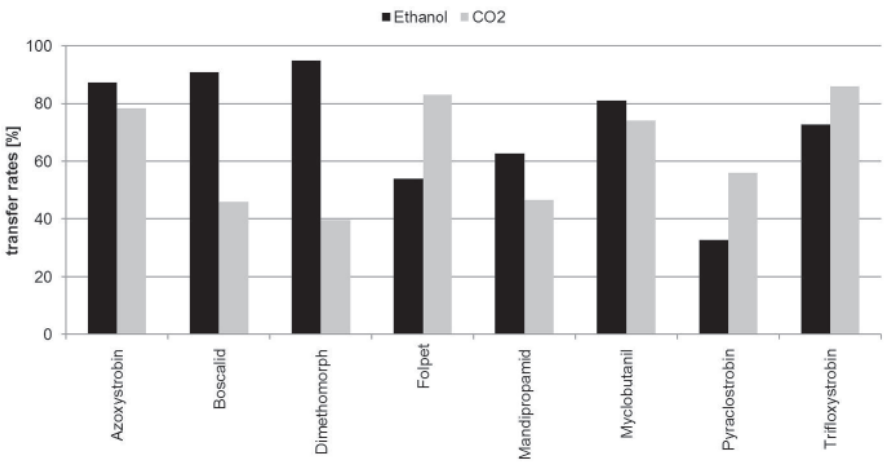

Fig. 2 **Average transfer rates of different active compounds during ethanol and carbon dioxide extraction (N = 3, except folpet: N = 2)**

Table 3 **Actual maximum residue levels (MRL), limits of quantitation (LOQ) and recovery rates (at 1 mg/kg) of all compounds covered by our multi-residue method**

Compound	MRL (2014) EU[12] [mg/kg]	MRL (2014) USA[13] [mg/kg]	MRL (2014) Japan[14] [mg/kg]	LOQ [mg/kg]	Recovery rate [%]
				LC-MS/MS	
Acetamiprid	0.1	–	–	0.1	116
Abamectin	0.05	0.2	0.2	0.05	108
Azoxystrobin	30	20	30	0.1	129
Boscalid	60	35	60	0.1	108
Carbofuran	0.05	–	10	0.05	107
Chlorantraniliprole	0.02	90	90	0.02	114
Cinidon-ethyl	0.1	–	0.1	0.1	65
Clothianidin	0.05	–	0.1	0.05	110
Cymoxanil	2	7	2	0.1	102
Diazinon	0.5	–	0.5	0.1	86
Dimethomorph	80	60	80	0.1	108
Fenpropimorph	10	–	0.1	0.1	116
Fenpyroximate	10	10	15	0.1	60
Flonicamid	2	7	5	0.1	123
Fluazifop-p-butyl	0.1	–	0.05	0.1	85
Fluopicolide	0.02	–	–	0.02	109
Hexythiazox	20	2	30	0.1	79
Imidacloprid	10	6	7	0.1	111
Linuron	0.1	–	0.02	0.1	85
Mandipropamid	50	50	50	0.1	101
Metalaxyl	10	20	10	0.1	117
Methidathion	0.1	–	5	0.1	82
Myclobutanil	2	10	10	0.1	78
Propamocarb	0.2	–	–	0.1	22
Propargite	100	100	100	0.1	64
Pymetrozine	15	6	15	0.1	42
Pyraclostrobin	15	23	15	0.1	81
Pyraflufen-ethyl	0.05	–	0.05	0.05	108
Spiroxamine	0.1	50	50	0.1	169
Tebuconazole	40	35	30	0.1	103
Terbuthylazine	0.1	–	–	0.1	125
Thiacloprid	0.1	–	–	0.1	110
Thiamethoxam	0.1	0.1	0.1	0.1	102
Tolylfluanid	1	–	50	0.1	79
Triadimenol	10	–	5	0.1	94
Zoxamide	0.05	–	–	0.05	108
				GC-MS/MS	
Bifenthrin	10	10	20	0.1	112
Captan	0.05	–	–	0.05	64
Cypermethrin	30	–	20	0.1	123
Etoxazole	15	7	15	0.1	91
Fipronil	0.01	–	0.002	0.02	80
Folpet	150	120	120	0.1	74
Lambda-cyhalothrin	10	10	10	0.1	108
Quinoxyfen	2	3	1	0.1	76
Spirodiclofen	40	30	40	0.1	82
Trifloxystrobin	30	11	40	0.1	86

polarity of the active compound and the solvent used for extraction. Figure 2 gives the transfer rates for some pesticides that have been detectable in unprocessed hops before extraction. These are the average values for three industrial scale processes (at least 50 t of unprocessed hops) used to make pure resin hop extract with carbon dioxide or ethanol. Only in the case of folpet was it necessary to use the average of two extraction batches as this active ingredient could always not be detected in one of the three raw hop samples for each extraction type.

As shown in Figure 2, the transfer rates deviate for different compounds. In most cases there was no clear dependence on the type of extraction solvent. Only for boscalid and dimethomorph the transfer rates resulting with carbon dioxide were significantly lower as compared to ethanol whereas for folpet and pyraclostrobin it was vice versa.

Nevertheless, the actual concentration of an active ingredient can be higher in the extract than in the corresponding unprocessed hops. This is due to the weight reduction during extraction. Depending on the variety and the extraction solvent the resulting yield by weight is in the range of approx. 15 % to 30 %. Thus, the relevant processing factor (100 divided by actual weight yield) must be taken into account when judging the suitability of an extract for trade. For hop extract, the MRL of hops is increased exactly by this factor. If the yield by weight is 30 % for example, the corresponding processing factor is 3.33. This means that the MRL of myclobutanil (see Table 3) for example is increased from “2 mg/kg” to “2 mg/kg x 3.33 = 6.7 mg/kg” in the resulting extract. Any concentration below this calculated limit is deemed for trading this extract.

Finally, information regarding the repeatability of our method and the inter-laboratory variability of hop residue analysis in general is provided in table 4.

The results of a collaborative trial organized in 2012 by an international working group for hop analysis called “Arbeitsgruppe Hopfenanalyse” (AHA) are presented. Nine laboratories (from Europe and USA) involved in the analysis of hop residues participated in this collaborative trial. As an example, the table shows the results for one pellet sample reported from our laboratory (“Hopsteiner”) as compared to the mean values (of all nine participants) or the ranges (minimum and maximum values), respectively. All participants detected a total number of eleven residues in this sample. Such a high number of detectable residues in an individual sample is very unusual; however, this sample was preselected, since worst case scenarios are preferable for the purpose of collaborative trials. All participants reported their average values based on duplicate analysis. The individual results of our duplicate analysis are presented to provide information on the repeatability of our method. For all compounds tested, our repeatability was less than +/– 10 % relative to our mean value, which is fully acceptable within the scope of residue analysis.

Moreover, our results correspond very closely to all total mean values. For some compounds, the whole (minimum to maximum) range is quite large. For example, for mandipropamid, the total

Table 4 **Results from a collaborative trial[a] for testing pesticide residues in one sample of hop pellets**

Compound	Results Hopsteiner [mg/kg][b]	Results of all 9 participants		
		Mean value [mg/kg]	Min. [mg/kg]	Max. [mg/kg]
Azoxystrobin	0.27/0.30	0.26	0.1	0.4
Boscalid	13.6/14.9	12.3	4.8	22.0
Dimethomorph	1.16/1.30	0.82	0.3	1.4
Folpet	5.76/5.95	4.31	3.0	5.9
Mandipropamid	6.00/6.19	6.83	1.4	16.5
Myclobutanil	0.83/0.89	0.58	0.1	0.9
Pyraclostrobin	1.07/1.18	1.22	0.4	2.5
Quinoxyfen	0.37/0.36	0.34	0.1	0.5
Spirodiclofen	1.16/1.34	1.19	0.3	1.5
Triadimenol	0.85/0.93	0.54	0.1	0.9
Trifloxystrobin	0.28/0.30	0.24	0.2	0.3

[a] organized by working group hop analysis.
[b] duplicate analyses

mean of all results reported from the nine participants is 6.83 mg/kg. The minimum and maximum values reported are 1.4 mg/kg and 16.5 mg/kg. This results in a (relative) range from 80 % lower as compared to the total mean or 240 % higher, respectively.

Usually a tolerance of +/– 50 % (relative) is accepted in pesticide analysis when judging the tradability of any plant material which is sold as food. This analytical tolerance for the reproducibility of residue analysis is based on the so-called "SANCO" document [16]. The example above clearly demonstrates again how difficult it is for hops to be in compliance with official standards which are applicable to other plants. The analytical methods of the other participants in this collaborative trial are all in-house methods. As far as we know, some of them are based on rapid multi-methods like "QuEChERS" which have been modified for hop analysis.

4 Conclusions

The presented multi-residue method has the capability of reaching LOQs of 0.1 mg/kg (or lower) in hops for a wide range of compounds which can be analysed by LC-MS/MS or GC-MS/MS. In addition to the scope indicated above, further pesticides may be detectable using the sample preparation procedure as described. We are currently working on the analysis of more than 20 additional compounds. Although the corresponding recovery rates are acceptable in most cases, the limits of quantification are still too high. The next challenge is to continue to improve and optimize analysis using LC-MS/MS or GC-MS/MS. For some compounds it is better to change the MS conditions, e.g. running LC-MS/MS in negative mode or GC-MS with chemical ionization. Through such modifications, the sensitivity for detection of some pesticides can be improved very efficiently (e.g. for detection of fipronil when running the negative MS mode). However, to a certain extent, our current equipment may represent a limiting factor. More sensitive MS detectors presently available are advantageous if the scope of this multi-residue method needs to be expanded. At this time, our approach is to employ individual methods for any relevant compound which has not yet been included in our multi-residue method. During the last two seasons, such individual methods were carried out for bromoxanil, diquat, dithianon, fosethyl-aluminium, MCPA and milbemectin (all permitted in Germany for hop cultivation but in the end not detectable in any sample from crop 2012). We are using different sample preparation procedures for single analysis of these compounds. In some cases, liquid extraction under acidic conditions is preferable. In addition, the MS conditions must be specifically modified for some of these active compounds as discussed above. Of course, our steady goal is to avoid individual methods and to analyse as many pesticides as possible using our multi-residue method.

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