Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

Direct injection high performance liquid chromatography coupled to data independent acquisition mass spectrometry for the screening of antibiotics in honey

Annie von Eyken [a], *Daniel Furlong* [a], *Samareh Arooni* [a], *Fred Butterworth* [b], *Jean-François Roy* [c], *Jerry Zweigenbaum* [d], *Stéphane Bayen* [a,*]

[a] *Department of Food Science and Agricultural Chemistry, McGill University, Canada*
[b] *Calgary Laboratory, Canadian Food Inspection Agency (CFIA), Calgary, AB, Canada*
[c] *Agilent Technologies, Saint-Laurent, QC, Canada*
[d] *Agilent Technologies, Wilmington, DE, USA*

ARTICLE INFO

Article history:
Received 28 September 2018
Received in revised form
19 December 2018
Accepted 27 December 2018
Available online 29 January 2019

Keywords:
Veterinary drugs
HPLC-Q-TOF-MS
Honey
Direct injection

ABSTRACT

The targeted analysis of veterinary drug residues in honey traditionally involves a series of extraction and purification steps prior to quantification with high performance liquid chromatography coupled to high resolution or tandem mass spectrometry. These steps, designed to separate the target analytes from interferences, are generally time-consuming and costly. In addition, traditional cleanup steps are likely to eliminate other compounds whose analysis could prove decisive in current or future assessment of the honey sample. Alternatively, direct injection without complex sample preparation steps has been introduced for the fast analysis of trace compounds in environmental and food matrices. The aim of this study was to develop a rapid method for the targeted analysis of 7 key veterinary drug residues in honey based on direct injection high performance liquid chromatography coupled to quadrupole time-of-flight, while simultaneously recording data-independent MS/MS (e.g. All Ions MS/MS data) for future re-examination of the data for other purposes. The new method allowed for the detection of the target residues at levels approximately 20–100 times lower than current regulatory limits, for a total analysis time of about 45 min. The recoveries (103–119%), the linearity ($R \geq 0.996$) and the repeatability (RSD $\leq$ 7%) were satisfactory. The method was then applied to 35 honey samples from the Canadian market. Residues of tylosin A, tylosin B, sulfamethazine and sulfadimethoxine were detected in 6, 9, 6 and 23% of the samples respectively, at levels below the regulatory limits in Canada. The possibility of adding a hydrolysis step to study sulfonamides in honey was tested, which provided good results for this family of compounds but lead to degradation of some of the other analytes. Finally, the non-targeted identification of several compounds was demonstrated as a proof of concept of future re-examination of All

* Corresponding author. Department of Food Science and Agricultural Chemistry, McGill University, 21111 Lakeshore, Ste-Anne-de-Bellevue, Quebec, H9X 3V9, Canada. Fax: +1 (514) 398 7977.
E-mail address: stephane.bayen@mcgill.ca (S. Bayen).

https://doi.org/10.1016/j.jfda.2018.12.013

Ions MS/MS data. This paper illustrates the capacity of this novel method to combine targeted and non-targeted screening of chemical residues in honey.

1. Introduction

The health and the well-being of honey bees is critical for both the natural environment and human food production systems. Indeed, 35% of the global food production is dependent on pollinators [1]. For some crops, up to 90% of the pollination is provided by honey bees [2]. In beekeeping, bees are exposed to three major diseases, namely the American foulbrood, the European foulbrood and nosemosis. In case of such threats, bee hives can be protected using antibiotics. Compounds such as tetracyclines, streptomycin, sulfonamides, tylosin, erythromycin, lincomycin, chloramphenicol, nitrofurans, nitroimidazoles, fluoroquinolones and fumagillin have been reported for bee protection [3]. In Canada, Maximum Residue Limits (MRLs) have been defined for the residues of oxytetracycline, tylosin and fumagillin in honey [4]. Health Canada has also defined and recommended some safe Working Residue Levels (WRLs) for a number of veterinary drugs approved for use in other species that may be detected in domestic or imported honey [5]. Table 1 describes the current MRLs and recommended WRLs for veterinary drug residues in honey in Canada.

The targeted analysis of veterinary drug residues in honey traditionally involves an extraction step prior to quantification with liquid chromatography coupled to mass spectrometry (LC-MS) or tandem mass spectrometry (LC-MS/MS). For example, Thompson et al. developed a method for the determination of lincomycin and tylosin in honey, based on solid-phase extraction (SPE) and liquid chromatography-atmospheric pressure chemical ionization mass spectrometry [6]. In their work, honey samples, previously liquefied in a water bath at 60 °C to remove wax and bulk debris, were dissolved with a Na_2CO_3/$NaHCO_3$ buffer. The resulting samples were loaded onto a C18 SPE cartridge. Using a sequence of washing steps (methanol/water 5:95 and then 30:70 (v/v) respectively), antibiotic residues were separated from the bulk of the sample matrix, notably sugars. A similar approach was adopted by Lopez et al. for the multiclass determination of antibiotic residues in honey, using SPE extraction and LC-MS/MS [7]. Recently, Orso et al. studied different extraction and cleanup methods for the determination of multiclass pesticides and antibiotics in honey samples, and the optimal conditions were determined to be homogenization with McIlvaine buffer followed by extraction with acetonitrile and cleanup with Florisil® using dispersive solid phase extraction, prior to LC-MS/MS analysis [8].

The above purification steps, designed to separate the target analytes from interferences, are generally time-consuming and costly. In addition, traditional cleanup steps are likely to eliminate other compounds whose analysis could prove decisive in current or future assessment of the honey sample (e.g. presence of other contaminants, chemical tracers, metabolites, etc.). Alternatively, direct injection without complex sample preparation steps has been introduced for the fast analysis of trace compounds in environmental and food matrices. For example, Bayen et al. reported a direct injection approach for the study of veterinary antibiotics in surface freshwater and seawater using liquid chromatography – electrospray ionization mass spectrometry (LC-ESI-MS) [9]. According to the authors, the recoveries obtained for the spiked compounds had an average of 95 ± 14% and 96 ± 28% for freshwater and seawater, respectively; linearity and limits of detection were acceptable for ecological risk assessment applications. Direct injection of seawater, which contains high concentrations of salts that could damage the instrument, was made possible by a post-column switch on the system that diverted the salt-containing solutions flushed out of the column to the waste. Similarly, Ciofi et al. recently investigated the applicability of direct injection of waste, surface, ground and drinking water samples into a LC-MS/MS system for the determination of perfluoro-alkyl acids [10]. Their method, based on the direct injection of the centrifuged water sample without any other treatment, was reported to have better sensitivity and repeatability than those achieved with other extraction methods, such as on-line SPE-LC-MS/MS. In the field of food analysis, Bayen et al. applied direct

Table 1 – MRLs and recommended WRLs for veterinary drug residues in honey in Canada [4,5].

Compound	Regulated concentration (μg/g)
Oxytetracycline	0.3 (MRL)
Tylosin (as tylosin A + B)	0.2 (MRL)
Fumagillin	0.025 (MRL)
Chlortetracycline	0.03 (WRL)
Erythromycin	0.03 (WRL)
Lincomycin	0.03 (WRL)
Streptomycin	0.0375 (WRL)
Sulfonamide drugs[a]	0.03 (WRL)
Tetracycline	0.075 (WRL)
Chloramphenicol	No MRL/WRL (Banned substance)
5-Nitrofuran compounds	No MRL/WRL (Banned substance)

[a] Only refers to the sulfonamide drugs listed in the "Table of Approved Administrative Maximum Residue Limits and Maximum Residue Limits" posted on Health Canada's website, which includes sulfacetamide, sulfabenzamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfaethoxypyridazine, sulfaguanidine, sulfamerazine, sulfamethazine, sulfanilamide, sulfanitran, sulfapyridine, sulfaquinoxaline and sulfathiazole.

injection LC-ESI-MS/MS for the analysis of pharmaceuticals and endocrine-disrupting chemicals in mussels and clams [11]. This "cleanup-free" approach, which relied on a simple solvent extraction with acetonitrile/methanol (50:50), was made possible using isotopically labeled surrogates to correct for matrix effects. As in the case of seawater analysis, a post-column switch on the LC-MS/MS system was used to remove potential interferences. Olmo-García et al. developed a method for metabolic profiling of phenolic compounds in olive oil using direct injection LC-ESI-MS [12]. The sample preparation was reduced to the dilution of olive oil in acetone, and the method was successfully validated and applied to the quantification of 21 phenolic compounds without any other step. In the context of honey analysis, direct injection LC-MS was successfully applied for the screening of various syrup adulterants and the presence of 10% sugar syrup in honey could be detected in less than 30 min [13]. "Dilute and shoot" approaches for honey have also been reported for the targeted determination of pesticides, veterinary drugs and other trace contaminants in honey prior to liquid chromatography coupled with time-of-flight MS, Orbitrap MS, and triple quadrupole MS [14–16].

The list of contaminants and toxins reported in food such as honey is continuously increasing, including new agrochemicals, emerging environmental pollutants and food contact material residues [17–19]. In this context, it appears essential to develop tools for the detection of currently unknown or unexpected contaminants. The need to further develop non-targeted methods has been highlighted by chemical risk assessment community to better characterize human exposure to chemicals [20], and to identify potential risk compounds in food matrices [21]. Among others, liquid chromatography coupled with high resolution mass spectrometry (HRMS) has emerged as a promising tool for the non-targeted analysis of food [21,22]. HRMS systems may be operated in full-scan mode or when using data-independent acquisitions for example, to obtain structural information about virtually all ionized compounds. In All Ions MS/MS, ions are fragmented in the collision cell without the selection of any specific precursor ion (data independent acquisition). Thus, unlike data-dependent acquisition modes, All Ions MS/MS provides fragmentation patterns for all the precursors. Perez-Ortega et al. applied All Ions MS/MS to the screening of over 625 multiclass organic food contaminants using high-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (HPLC-Q-TOF-MS), and found that this acquisition mode was more appropriate for the large-scale screening than the classic product ion scans because it provided excellent fragmentation information for confirmatory purposes for a theoretically unlimited number of compounds [23]. When comparing the performance of HRMS and All Ions MS/MS, the latter was found to preserve full-scan acquisition flexibility and to have the benefits of acquiring all the information all the time without the time window boundaries of targeted MS/MS mode and without the potential loss of sensitivity of non-targeted MS/MS [23]. Therefore, All Ions MS/MS mode could be seen as a combination of the mass resolution capabilities of HRMS and the identification capabilities of MS/MS spectra obtained without compromising the detection of trace compounds in complex matrices. As direct injection allows for a minimal modification of the sample, its coupling with high resolution mass spectrometry is expected to provide a broad screening of samples' composition. To date, direct injection in combination with HRMS in the All Ions MS/MS mode for the screening of food contaminants has not been reported.

The aim of this study was to develop (i) a rapid method for the targeted analysis of seven veterinary drug residues in honey based on direct injection HPLC-Q-TOF-MS, while (ii) simultaneously recording non-targeted information (fast high-resolution MS scans combined with All Ions MS/MS) for future re-examination of the data (e.g. for exposure assessment). Seven target compounds were selected to explore the performances of the approach for different families of veterinary drugs related to beekeeping (i.e. macrolides, lincosamides, nitrofurans and sulfonamides) [3]. They were all reported to be of concern due to their toxicity, probability of antibiotic resistance, frequency of dosing or evidence of detectable residues [24]. It should be noted that sulfonamides are known to bind to sugars in honey, and acid hydrolysis is commonly required in order to liberate them and to study the total amount in honey (free + bound) [25]. Therefore, the presented approach was tested with and without an acid hydrolysis step to study sulfonamides in honey samples. The data acquired with the method developed in the present study could be used in the future to re-examine for the presence of currently unknown contaminants, or to identify some shift in the quality of honey over time. The novelty of this study is the use of direct injection combined with HRMS in the All Ions MS/MS mode for the targeted and non-targeted screening of food contaminants.

2. Materials and methods

2.1. *Chemicals and reagents*

Analytical standards (tylosin A (CAS Number 1404-69-0), lincomycin (CAS Number 859-18-7), furazolidone (CAS Number 67-45-8), sulfamethoxazole (CAS Number 723-46-6), sulfadimethoxine (CAS Number 122-11-2) and sulfamethazine (CAS Number 57-68-1)) were purchased from Sigma–Aldrich (St Louis, MO, USA). Tylosin B (CAS Number 11032-98-7) was purchased from Toku-E (Bellingham, WA, USA). Labelled internal standards, D3-diphenhydramine (CAS Number 170082-18-5) and D3-6-acetylmorphine (CAS Number 152477-90-2), were purchased from Cerilliant (Round Rock, TX, USA). All standards were of analytical grade. HPLC grade solvents (water, methanol, acetonitrile, acetone and 2-propanol), as well as LC/MS grade formic acid were all purchased at Fisher Chemical (Pittsburgh, PA, USA). Hydrochloric acid (37%) and D-(+)-glucose (≥99.5%) were purchased from Sigma–Aldrich.

2.2. *Honey samples*

Twenty-six honey samples (H1-H26) were purchased from different stores and farmers' markets in the Montreal and Calgary regions (Canada) in May 2016. Details from the samples are presented in Table S1. They were all unpasteurized and of various prices and types (i.e. non-organic, organic

farming, different colors and different floral origins). Nine additional selected honey samples (H27-H35) were obtained from the Canadian Food Inspection Agency (CFIA) in December 2016. These nine samples had been earlier analyzed by the CFIA Calgary Laboratory using class-specific multi-residue methods developed and validated in-house, and were used to test the performance of the present new method. All samples were transferred from their original container to 40 mL amber glass vials and kept in the freezer at −18 °C until analysis.

2.3. Sample preparation

2.3.1. Method A: sample preparation without acid hydrolysis

Sample preparation was adapted from Du et al. [13]. Approximately 0.2 g of honey was weighed in a glass conic tube and 2 mL of a mixture of acetonitrile and water (1:1) was added. Samples were vortexed for about 2 min, or until the honey was completely dissolved, and then filtered through a 0.22 μm PTFE filter from Chrom4 (Thüringen, Germany). Before injection into the HPLC, the extract was further diluted with water to a final concentration corresponding to 1% of honey, and 50 μL of a 0.4 μg/mL mixture of the two internal standards was added. These internal standards were not used for quantification in this study but were spiked to provide a reference for sensitivity and retention time, necessary for the future non-targeted data treatment.

2.3.2. Method B: sample preparation with acid hydrolysis

Approximately 0.2 g of honey was weighed in a glass conic tube and 1 mL of HCl 2M was added. Samples were vortexed for about 5 min, and then they were allowed to sit at room temperature for 1 h. Samples were then filtered, diluted and spiked with internal standards as above in Method A.

2.4. Method validation

In a preliminary study, three honey samples without any detectable amounts of the target antibiotics were selected as matrix blanks (H7, H18 and H26). They were from different floral and geographical origins, as well as different colours and farming methods, representing the variability of matrices within the study. To establish the absolute recoveries and the linearity of both Methods A and B, these samples were spiked with 50 μL of standard of the target antibiotic analytes in methanol at 7 levels in the 0.004–2 μg/g range before sample dilution. For Method B, samples were allowed to sit overnight at room temperature after spiking in order for the sulfonamides to react with sugars [26]. To study the matrix effect, the native antibiotic standards were spiked directly on the 1% honey sample ready for LC-QTOF analysis, at 7 levels in the 0.04–20 ng/mL range in the injected sample, corresponding to 0.004–2 μg/g in honey. Procedural blanks (n = 10) were analyzed and used to derive the limits of detection (3σ). For repeatability studies, 5 replicates of three spiked honey samples were analyzed.

To further investigate the effect of acid hydrolysis on sugar-sulfonamide conjugates, additional honey samples were spiked with the 3 sulfonamides (0.2 μg/g) and left to sit overnight for the sulfonamides to bind with sugars [27]. Samples were then prepared using both Method A and Method B (n = 3 each). Signals corresponding to glucose-sulfonamide conjugates were identified in the chromatograms through the comparison with three standard mixtures rich in glucose-sulfonamide conjugates. These mixtures were then prepared by mechanochemical mixing of an equimolar mixture of glucose and individual sulfonamide in a Retsch Mixer Mill (MM 400 Newtown, PA, US) at room temperature using two stainless steel balls and a frequency of 30 Hz for 30 min. The reaction mixture for each sulfonamide was then suspended in water and filtered, and the residue was analyzed after dilution in water/methanol 95:5 (v/v).

2.5. Instrument analysis

Samples were analyzed using a 1290 series LC system from Agilent Technologies (Santa Clara, CA, USA) equipped with an InfinityLab Poroshell 120 Phenyl Hexyl (3.0 × 100 mm, 2.7 μm) column fitted with an InfinityLab Poroshell 120 EC-C18 (3.0 × 5 mm, 2.7 μm) guard column, both from Agilent Technologies. The mobile phase consisted in a mixture of water with 0.1% formic acid (solvent A) and methanol with 0.1% formic acid (solvent B), at a flow rate of 0.2 mL/min. The mobile phase gradient profile was as follows: 1 min 5% B, from 1 to 15 min gradient to 100% B, from 15 to 20 min 100% B, from 20 to 20.10 min gradient to 5% B and from 20.10 to 25 min 5% B. The injection volume was 20 μL and the column temperature was set to 20 °C. The LC system was coupled to a 6545 series Q-TOF from Agilent Technologies (Santa Clara, CA, USA) equipped with a Dual AJS ESI ion source operating in positive ionization mode. Drying gas temperature was 325 °C with a flow of 5 L/min, sheath gas temperature was 275 °C with a flow of 12 L/min, the pressure on the nebulizer was 20 psi, the capillary voltage was 4000 V, the fragmentor voltage was 175 V, the skimmer voltage was 65 V and the nozzle voltage was 2000 V. All Ions MS/MS data was collected as MS scans between *m/z* 100 and 1700 at a scan rate of 3 spectra/s for four different collision energies (0, 10, 20 and 40 eV). A diode-array detector (DAD; scan range: 190–640 nm with a 2 nm step and a slit of 4 nm) coupled to the HPLC-Q-TOF-MS system was also used for the study of the diversion of elution to waste. Samples were kept at 4 °C in the multisampler compartment.

2.6. Data treatment

Instrument response linearity was calculated for each compound as the relative standard deviation (RSD) of the response factors (RF) of the seven calibration curve standards (ranging 0.08–40 ng/mL). Antibiotic concentrations were calculated using the Agilent Mass Hunter Workstation Software – Quantitative Analysis B.07.01, for three different *m/z* extraction window values (±5, ±10 and ±20 ppm). Extraction window values were selected to represent the range of values used by other authors in similar targeted and non-targeted studies [28–30]. Quantification was done based on external calibration, and peaks with a signal-to-noise ratio below 10 were considered below the limit of quantification. The following mass-to-charge ratios were used for quantification: 916.5270 for tylosin A, 772.4483 for tylosin B, 407.2216 for lincomycin, 226.0464 for furazolidone, 254.0599 for sulfamethoxazole,

279.0916 for sulfamethazine and 311.0814 for sulfadimethoxine. Matrix effect, recovery, instrument linearity, method linearity, repeatability, instrument detection limit (IDL), method detection limit (MDL) and limit of quantification (LOQ) were calculated for each compound for each of the three *m/z* extraction window values. Matrix effect (ME) and recovery (RE) were calculated according to the equations proposed by Matuszewski by comparing the response of each compound in samples spiked before dilution (RE), after dilution (ME) and in solvent [31]. The overall method linearity was assessed from the Pearson coefficient of the linear correlation between the experimental and theoretical spiked concentrations. Repeatability was assessed from the RSD obtained for five replicates of three spiked honey samples. IDL was calculated as the concentration leading to a signal-to-noise ratio (S/N) of 3, derived from the S/N of the lowest standard of the calibration curve. MDL was calculated as 3σ of the signals of 10 procedural blanks around the retention time of each compound. LOQ was calculated as 3.3 times the MDL.

Two-way analysis of variance (ANOVA) tests were performed using SigmaPlot v13.0 (Systat Software Inc) to compare the performances of the method (matrix effect, recovery, repeatability, method linearity and MDL) obtained for the different *m/z* extraction window values.

After confirmation of the linearity of the method with three matrix matched curves, antibiotics were quantified in all the 35 honey samples based on the standard addition method with one single level of spiking corresponding to a concentration of 0.2 μg/g in honey. This standard addition, which was within the range of linearity, was done to the already diluted extract to compensate for the matrix effect of each honey sample [32].

For the non-targeted applicability of the method, the honey samples were screened for the 7 veterinary drugs and other compounds using Agilent Mass Hunter Workstation Software – Qualitative Analysis B.07.00. The chromatogram was explored using the algorithm Find By Formula, using All Ions MS/MS and a customized database of honey-related compounds created with Personal Compound Database and Library software (PCDL) for LC/TOF. For the library screening, match tolerance was set to ±5 ppm and expansion values for chromatogram extraction at ± 10 ppm.

3. Results and discussion

3.1. Diversion of elution to waste

Introducing a high amount of sugars, the main components of honey, into the ion source could increase needs for cleaning and maintenance. To circumvent this issue, a post-column switch was used to divert the fraction containing the sugars directly to waste. This diversion of elution to waste to avoid the introduction of high amounts of matrix-related highly-polar compounds into the ionization source was successfully applied by other authors during the analysis of contaminants in seawater and seafood using direct injection [9,11]. Fig. S1 shows the total wavelength chromatogram obtained for a honey sample spiked with the mixture of the seven veterinary drug residues. The first peak at around 2.5 min corresponded to the sugars, which are expected to elute early in reversed-phase chromatography. From a targeted point of view, the first compound of interest (lincomycin, Log $K_{OW} = 0.86$ [33]) did not elute until 9 min, so the first 0.6 mL (3 min) eluting out of the column were directly sent to waste after which the post-column switch position changed to the ion source. From a non-targeted point of view, since most contaminants are expected to be less polar than the first-eluting matrix-related salts and sugars, it was decided to compromise the possible loss of a minority of molecules of interest for the lifetime of the ion source and the throughput capabilities of the method.

3.2. Method performance (Method A – sample preparation without acid hydrolysis) and m/z extraction window selection

Instrument linearity, IDL, matrix effects, MDL, LOQ, recoveries, method linearity and repeatability are presented in Table 2 for each of the seven analytes and each *m/z* extraction window. RSD values of the RF of the calibration curve standards were used to assess instrument linearity. RSDs were generally below 30% with the only exception of tylosin A at ± 5 ppm, which presents a RSD of 44%. In general, as the *m/z* extraction window decreased, so did the signal intensities. This may result in a decreased precision of the RF, especially for the lowest concentrations, and poorer instrument linearity. IDLs were in the range of 0.002 and 0.017 ng/mL for the three *m/z* extraction windows tested.

Matrix effects obtained for a ±5 ppm m/z extraction window were significantly different than those at ± 10 and ±20 ppm (ANOVA, $P = 0.014$). According to the equation used, an absence of matrix effect corresponds to a value of 100%. Values below 100% correspond to matrix suppression, while values above 100% correspond to matrix enhancement. Generally, matrix effects are considered to be mild when the values are between 80 and 120%, medium between 50 and 80% or 120 and 150%, and strong for values below 50% or above 150% [34]. In this study, even though the results obtained for a ±5 ppm m/z extraction window were statistically different than those at ± 10 and ±20 ppm, most of the matrix effect values were mild and only two cases of medium matrix effects were noted, for furazolidone at ± 5 ppm (ME = 63%) and sulfamethazine at ± 20 ppm (ME = 123%). Matrix effects in this study were overall lower than those reported by Orso et al. for veterinary drugs in honey using a traditional extraction approach with SPE. In their study, strong matrix suppression was reported for tylosin A (ME = 23.4%) and strong matrix enhancement was observed for sulfamethoxazole and sulfadimethoxine (ME = 181.0% and ME = 183.8% respectively) [8]. In the present study, signal suppression was noted for tylosin A, lincomycin, furazolidone and sulfamethoxazole at all *m/z* extraction window values. However, Orso et al. reported signal suppression for tylosin A only, while mild to strong signal enhancements were recorded for lincomycin, furazolidone, sulfamethoxazole, sulfamethazine and sulfadimethoxine [8]. On the other hand, Lopez et al. reported signal suppression for lincomycin in honey using SPE extraction [7]. In the present study, matrix effects varied among honey samples, as illustrated by the standard deviation on the ME values. This suggests that matrix effects are strongly

Table 2 – Method performance (Method A) for the seven targeted veterinary drug residues for *m/z* extraction windows of ± 5, ± 10 and ± 20 ppm.

Parameter	Extraction window	Tylosin A *m/z* 916.5270 RT[b] 14.64 min	Tylosin B *m/z* 772.4483 RT 13.91 min	Lincomycin *m/z* 407.2216 RT 9.17 min	Furazolidone *m/z* 226.0464 RT 11.69 min	Sulfamethoxazole *m/z* 254.0599 RT 11.57 min	Sulfamethazine *m/z* 279.0916 RT 10.73 min	Sulfadimethoxine *m/z* 311.0814 RT 12.96 min
Instrument	±5 ppm	44	17	9	24	13	25	15
Linearity (RSD	±10 ppm	8	12	9	25	12	10	13
% of RF)	±20 ppm	6	10	9	26	14	9	14
IDL (ng/mL)	±5 ppm	0.007	0.009	0.002	0.003	0.012	0.002	0.002
	±10 ppm	0.012	0.007	0.001	0.003	0.010	0.002	0.002
	±20 ppm	0.011	0.009	0.002	0.003	0.017	0.002	0.002
Matrix effect (%)[a]	±5 ppm	97 ± 37	108 ± 14	82 ± 10	63 ± 26	86 ± 17	97 ± 21	84 ± 23
	±10 ppm	98 ± 8	111 ± 9	82 ± 10	98 ± 31	88 ± 17	115 ± 23	102 ± 17
	±20 ppm	98 ± 8	111 ± 10	85 ± 12	92 ± 24	94 ± 18	123 ± 42	102 ± 21
MDL (μg/g honey)	±5 ppm	0.0025	0.0015	0.0008	0.0016	0.0022	0.0009	0.0005
	±10 ppm	0.0023	0.0015	0.0003	0.0014	0.0018	0.0007	0.0005
	±20 ppm	0.0017	0.0017	0.0008	0.0020	0.0023	0.0009	0.0008
LOQ (μg/g honey)	±5 ppm	0.0084	0.0048	0.0027	0.0054	0.0072	0.0029	0.0016
	±10 ppm	0.0076	0.0050	0.0011	0.0047	0.0060	0.0025	0.0018
	±20 ppm	0.0055	0.0056	0.0026	0.0067	0.0077	0.0030	0.0025
Recovery (%)[a]	±5 ppm	107 ± 39	112 ± 21	117 ± 17	126 ± 36	110 ± 30	111 ± 33	102 ± 47
	±10 ppm	109 ± 10	115 ± 12	119 ± 13	103 ± 16	115 ± 23	118 ± 21	108 ± 12
	±20 ppm	112 ± 13	115 ± 12	120 ± 10	114 ± 24	113 ± 18	123 ± 44	113 ± 20
Method linearity	±5 ppm	0.9736	0.9983	0.9995	0.9993	0.9987	0.9990	0.9975
(R)	±10 ppm	0.9980	0.9988	0.9996	0.9963	0.9987	0.9990	0.9987
	±20 ppm	0.9979	0.9984	0.9996	0.9960	0.9986	0.9990	0.9987
Repeatability	±5 ppm	37	5	6	24	6	11	20
(RSD %)	±10 ppm	4	4	5	5	6	7	7
	±20 ppm	4	4	5	5	5	7	7

[a] Matrix effects and recovery values are presented as mean of all concentration levels ± standard deviation (n = 21).
[b] RT = Retention Time.

dependent on the characteristics of individual honey samples. The application of an average matrix effect may therefore inaccurately correct for the matrix effects, and we would recommend the assessment of the matrix effects for each honey sample.

MDLs were not statistically different using ±5, ±10 or ±20 ppm of *m/z* extraction window (ANOVA, P = 0.166). With values in the range of 0.0003–0.0025 μg/g in honey, these MDLs correspond to the detection of 0.6–5 pg of antibiotic injected. These MDLs are in the same range as those reported by other authors for organic contaminants using the direct injection approach in other matrices [10]. These MDLs are also in the same range as those reported for veterinary drugs and pesticides in honey using conventional extraction approaches [8,35]. Most importantly, our MDLs are around 20 to 100 times lower than their respective regulatory limits in Canada, the MRLs or WRLs for these substances in honey (Table 1). No MRLs have been set up for these antibiotics in honey in other countries such as Australia, the European Union or the United States [36–38]. LOQ were in the range of 0.0011–0.0084 μg/g in honey, which is around 25 times lower than the regulatory limits.

All the recoveries were within the 80–120% acceptable range, with the only exception of furazolidone at ± 5 ppm and sulfamethazine at ± 20 ppm with values of 126 and 123%, respectively. These values were in the same order as those reported by other authors for pesticides and veterinary drugs in honey, where the recoveries generally ranged 80–120% with a few exceptions above or below this range [8,15]. There was no statistical difference (ANOVA, P = 0.591) amongst the recoveries obtained for different *m/z* extraction windows. However, since all the recoveries for the ±10 ppm m/z extraction window were systematically <120%, this value was selected for the rest of this study.

Regarding method linearity, results showed no significant difference for any of the compounds at all three *m/z* extraction window values (ANOVA, P = 0.462). Pearson coefficients were between 0.9960 and 0.9996 with the only exception of tylosin A, which presented a slightly lower R value of 0.9736 at ± 5 ppm. These high Pearson coefficients were similar or higher than those reported for honey or for other matrices using the direct injection approach [8,12]. For this reason, the method is considered linear.

With regards to repeatability, there was a clear difference between the results at ± 5 ppm and at ± 10 and ±20 ppm, and RSD values of up to 37% for tylosin A were recorded at ± 5 ppm. Repeatability for ±10 and ±20 ppm were all below 10%. This difference was confirmed by the statistical tests (ANOVA, P = 0.014). As commented before with the instrument linearity, this can be due to the fact that the signals are generally smaller at ± 5 ppm in comparison with ±10 and ±20 ppm, and this may affect repeatability. At ± 10 and ± 20 ppm, the RSD values obtained in this study were in the same order or lower than those reported by other authors [7,12].

In conclusion, a *m/z* extraction window of ±10 ppm was selected for the treatment of the honey sample data as satisfactory performances were obtained at that value. This value has also been used by other authors doing similar studies on organic contaminants and metabolites in food with HPLC-HRMS [23,39].

Fig. 1 shows the overlapped extracted ion chromatograms of the 7 analytes spiked in one of the honey samples at a concentration of 0.2 μg/g, corresponding to the MRL of tylosin in honey, extracted using a *m/z* extraction window of ±10 ppm. Chromatographic peaks for all 7 compounds can be clearly identified with minimal background interferences with the present method. Altogether, satisfactory performances were obtained for 7 key veterinary compounds with the present method, with the added benefits of (i) a much shorter analysis time compared to current methods, and (ii) recording non-targeted information for future re-assessment of the data. In addition, this direct injection approach satisfies some of the requirements of green analytical chemistry, since it is a direct analytical technique that avoids sample treatment, it has minimal sample size and reduced reagent consumption in comparison with the traditional methods of honey analysis, derivatization is avoided, and it is a multi-analyte method [40].

3.3. *Application to honey samples*

The above optimized method was applied to 35 honey samples collected from the Canadian market. Most of the results were below the MDL, and only tylosin A, tylosin B, sulfamethazine and sulfadimethoxine were detected in some samples. The concentrations of these four antibiotics in the samples they were detected are shown in Table 3. As mentioned earlier, in absence of hydrolysis, these concentrations correspond to the free species of the compounds in honey. The highest values were obtained for tylosin B, with concentrations up to 0.0703 μg/g. None of the samples contained residues of tylosin (A + B) above the MRL of 0.2 μg/g set in Canada. Similarly, the levels of free sulfamethazine and sulfadimethoxine were below their respective recommended WRL in Canada (0.03 μg/g).

In their most recent National Chemical Residue Monitoring Program (NCRMP, 2013–2014 report), the CFIA reported the occurrence of tylosin A in 10.05% of the domestic honey samples and in 6.10% of the imported ones. The frequency of detection for tylosin B was 19.74 and 14.71% for domestic and imported honey, respectively [41]. In this study, 6% of the samples were positive for tylosin A and 9% for tylosin B, so the

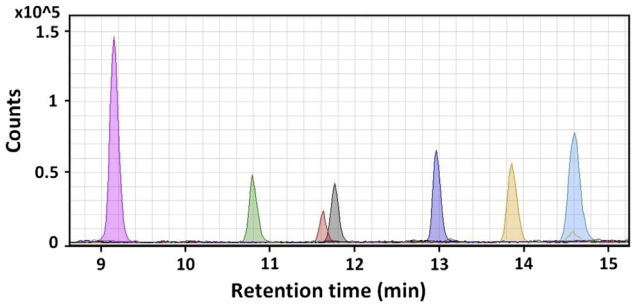

Fig. 1 – Overlapped extracted ion chromatograms for the 7 antibiotics in sample H7 spiked at a concentration corresponding to 0.2 μg/g in honey (Sample preparation Method A). Order of elution: lincomycin, sulfamethazine, sulfamethoxazole, furazolidone, sulfadimethoxine, tylosin B and tylosin A.

Table 3 – Concentration (μg/g) of tylosin A, tylosin B, sulfamethazine and sulfadimethoxine in the honey samples they were detected, at a *m/z* extraction window of ±10 ppm.

Sample	Tylosin A	Tylosin B	Sulfamethazine[c]	Sulfadimethoxine[c]
1	ND[a]	0.0021	ND	ND
6	ND	ND	ND	0.0045
7	ND	ND	ND	0.0039
8	ND	ND	ND	0.0042
11	ND	ND	ND	0.0017
14	ND	ND	<0.0023[b]	0.0035
15	ND	ND	ND	0.0074
18	ND	ND	ND	0.0022
29	ND	ND	ND	<0.0017[b]
30	ND	ND	<0.0023[b]	ND
32	<0.0076[b]	0.0221	ND	ND
35	0.0176	0.0703	ND	ND

[a] ND = non-detected.
[b] Compounds detected at concentrations below the LOQ.
[c] Concentration of sulfamethazine and sulfadimethoxine refers to the free form of these compounds in honey.

rates of detection of these compounds are similar to those found by the CFIA. No sulfonamide antibiotics were reported by the CFIA in their 2013–2014 report, but sulfadimethoxine and sulfamethoxazole had been detected in 4.35 and 3.26% of the imported honeys, respectively, in the 2012–2013 NCRMP CFIA Report [42]. In the present study, free sulfadimethoxine was detected in 23% of the honeys, so the present rate of detection of this compound was higher than that reported by the CFIA in 2012–2013.

Tylosin A is the main component of the commercial tylosin formulation applied to honeybees [43]. The ratio of mass concentrations of tylosin A to tylosin B in the present study was measured to be 0.16 and 0.25 for samples 32 and 35 respectively. These values are lower than the overall average reported by Thompson et al. of 1.2 ± 0.2 in honey in Canada [44]. Tylosin A degrades into tylosin B in honey, suggesting that the ratio of their concentrations can decrease over time [43,45]. Bohm et al. studied tylosin A and tylosin B in honey following the application of tylosin A tartrate on honeybees, and their ratio decreased from 4.31 after 3 days of application to 0.73 after 52 days [46]. As a consequence, the World Health Organization Expert Committee on Food Additives (JECFA) listed tylosin B as a major end product of tylosin A in honey, and recommended to take into account both tylosin A and B when considering food safety [47].

3.4. *Comparison with a standard method*

The nine samples provided by CFIA had been previously tested for their content of tylosin A using the official CFIA method ACC-066. These results were used as a blind comparison to test the performance of the present new method. As shown in Table 4, the results of this study matched the positive and negative results reported by the CFIA. Moreover, the concentrations of tylosin A measured with the present method were comparable to those obtained with the official methods for the two positive samples. In conclusion, this method is expected to perform well for the monitoring of veterinary drugs in honey, with low chances of misclassification of samples (false negatives or false positives).

3.5. *Performances of the method including an acid hydrolysis step (Method B)*

The addition of an acid hydrolysis step (Method B) was tested to assess the total content of sulfadimethoxine, sulfamethazine and sulfamethoxazole in honey. Method performances are presented in Table S2 and the direct injection method following an acid hydrolysis gave overall satisfactory results for the three sulfonamides. Matrix effects were however greater for sulfadimethoxine and sulfamethoxazole, and precision was slightly poorer (19–23%) for sulfonamides with respect to direct injection method without hydrolysis. It is important to highlight that tylosin A and furazolidone were detected in honey samples treated by acid hydrolysis. Low recoveries for tylosin A were expected for Method B, since tylosin A has been reported to degrade under acidic conditions [48].

In order to confirm the effect of acid hydrolysis on sugar-sulfonamide conjugates, additional spiked honey samples (H7, H18 and HX26) were equilibrated overnight and prepared using both Methods A and B. Fig. 2A–C shows the peaks of sulfamethoxazole, sulfamethazine and sulfadimethoxine

Table 4 – Concentration of tylosin A in the CFIA honey samples according to the reference method and in the present method (Method A), expressed as μg/g.

Sample	Reference method (CFIA ACC-066)	Present method (MDL = 0.0023 μg/g)
27	ND	ND[a]
28	ND	ND
29	ND	ND
30	ND	ND
31	ND-	ND
32	0.0060	<0.0076[b]
33	ND	ND
34	ND	ND
35	0.0136	0.0176

[a] ND = non-detected.
[b] Detected at a concentration below the LOQ.

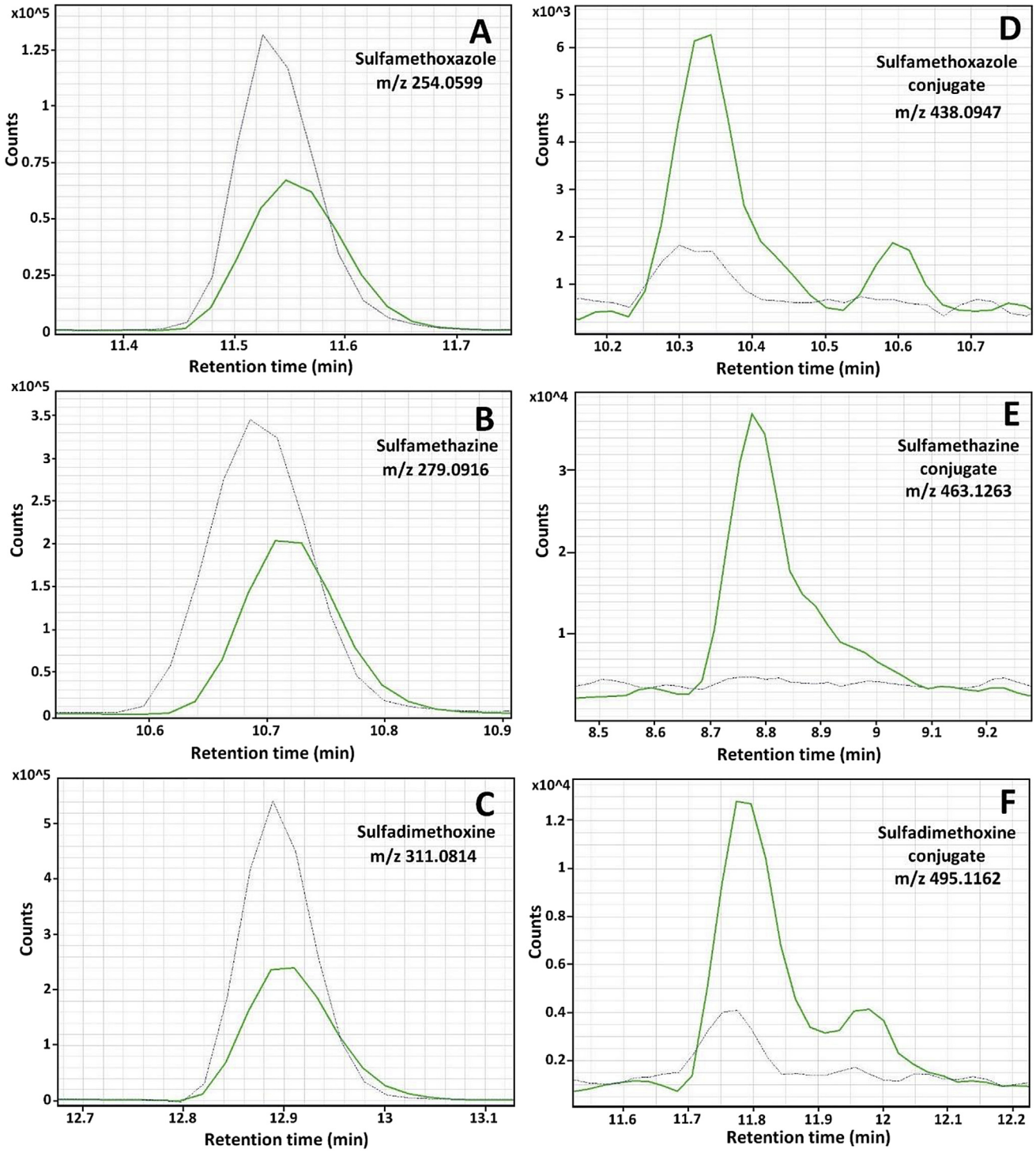

Fig. 2 – Extracted ion chromatograms of sulfamethazine (A), sulfamethoxazole (B), sulfadimethoxine (C), glucose-sulfamethazine conjugate (D), glucose-sulfamethoxazole conjugate (E) and glucose-sulfadimethoxine (F) in sample H18 spiked with all 7 target veterinary drugs at a level corresponding to 0.2 μg/g in honey, which was extracted with hydrolysis (blue dotted line) and without hydrolysis (green line). The extracted ions in A-C and D-D were $[M+H]^+$ and $[M+Na]^+$, respectively.

spiked in sample H18 and extracted with both methods. In all three samples, the amount of sulfonamides detected following acid hydrolysis (Method B) was greater than without hydrolysis (Method A), confirming the release of conjugated sulfonamides under acidic conditions. In parallel, the mass spectra and the retention time of glucose-sulfonamide conjugates were determined in the HPLC-QTOF-MS chromatograms obtained for the three sulfonamide-glucose standard mixtures (Fig. S2). This information was then used to interpret the chromatograms obtained for the three spiked honey

samples equilibrated overnight and extracted with and without acid hydrolysis. As observed in Fig. 2D–F, the glucose-sulfonamide conjugates were detected in honey samples injected in the HPLC-QTOF-MS without hydrolysis, but not after acid hydrolysis. The attribution of this signal to a glucose-sulfonamide conjugate was confirmed by comparison of the retention time (Fig. S2) and the All Ions MS/MS spectra of the synthesized conjugate. Indeed, the $[M+Na]^+$ ion (*m/z* 438.0947 for glucose-sulfamethoxazole conjugate, *m/z* 463.1263 for glucose-sulfamethazine conjugate and *m/z* 495.1162 for glucose-sulfadimethoxine conjugate) and one characteristic fragment (*m/z* 254.0594 for glucose-sulfamethoxazole conjugate, *m/z* 186.0330 for glucose-sulfamethazine conjugate and *m/z* 156.0764 for glucose-sulfadimethoxine conjugate) were observed for each conjugate in the honey samples and in the standard mixture with

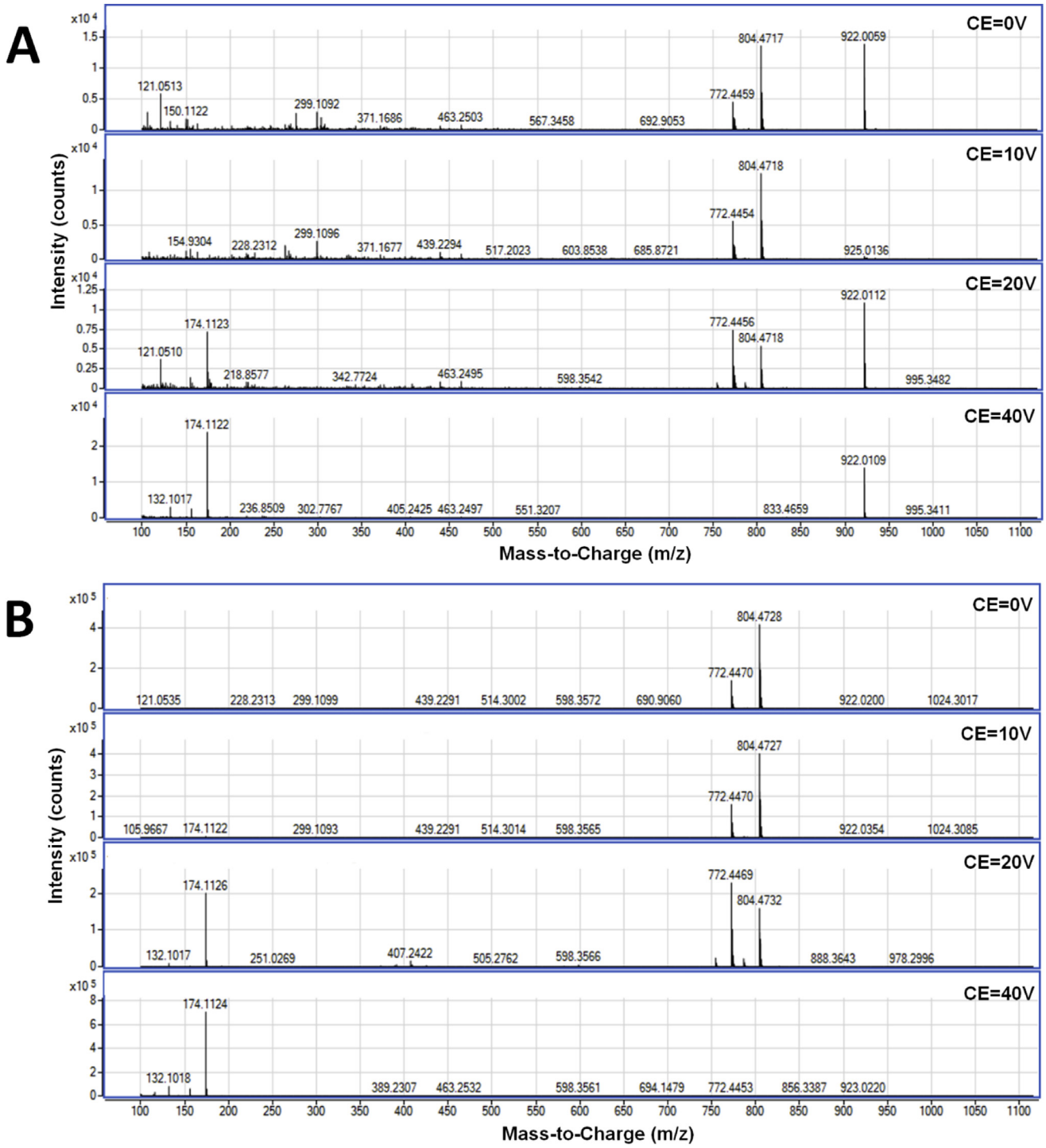

Fig. 3 – All Ions MS/MS spectra of tylosin B for different collision energies (CE). A: in honey sample H35. B: in an analytical standard (20 ng/mL in methanol/water).

similar relative abundances. To the best of our knowledge, this is the first time these glucose-sulfonamide conjugates have been reported in honey, and this was made possible through the coupling of direct injection and HRMS and the interpretation of All Ions MS/MS data. The detection of these conjugates in honey has very promising applications, as it opens the doors to including sulfonamides in multi-residue and non-targeted methods without compromising the stability of other analytes (e.g. tylosin A) with an extra acid hydrolysis step. Further studies are required to fully validate the quantification of these conjugates.

3.6. *Application of the method for the non-targeted identification of contaminants in honey*

The ultimate application of this method is to provide non-targeted information for future re-examination of the data for other purposes (e.g. in the context of exposure assessment).

To first illustrate the capacity of this approach in identifying unknown compounds in honey based on All Ions MS/MS, the data obtained for one of the honey matrix blanks spiked with the seven target analytes at a concentration of 2 μg/g were treated using Agilent Mass Hunter Workstation Software – Qualitative Analysis B.07.00. The chromatogram was explored using the algorithm Find By Formula, using All Ions MS/MS data and a customized database of honey-related compounds created with Personal Compound Database and Library software (PCDL) for LC/TOF. This algorithm was applied by other authors for identification of suspects using HPLC-QTOF-MS [49]. All 7 analytes were successfully identified in this sample with total scores above 70%, confirming the non-targeted capability of the approach. The total score reflects the probability that a feature being correctly identified as a specific compound, being a score of 100% a perfect fit [22]. As an example, Fig. 3 shows the All Ions MS/MS spectra of tylosin B in sample H35 and in the standard. The characteristic $[M+H]^+$ ion of tylosin B can be observed in the honey sample at 0, 10 and 20 V with an *m/z* of 772.4459, 772.4454 and 772.4456 respectively (exact mass: 772.4483). Its main fragment $[C_8H_{16}NO_3]^+$, commonly reported by others [50], can be observed at a CE of 20 and 40 V with an *m/z* of 174.1123 and 174.1122 respectively (exact mass: 174.1130).

To further demonstrate the non-targeted applicability of the method beyond the veterinary drugs of interest, the 35 honey samples were screened for other compounds related to beekeeping using the same Find By Formula Algorithm and different databases of honey-related compounds created with PCDL LC/TOF. Nine out of the 35 honey samples were found to contain hydroxymethylfurfural (HMF), a heat-induced contaminant commonly found in honey samples that had been submitted to heat treatments or a long storage time [51]. Once the exact mass of this compound was identified as HMF by the Find By Formula Algorithm with a score above 70%, the confirmation of its identity was carried out in the same way as for tylosin B by comparing the All Ions MS/MS spectra with a standard. The $[M+H]^+$ ion of HMF (exact mass: 127.0395) and one of its characteristic fragments (exact mass: 109.0289) were observed with similar relative intensity in the honey samples and in the standard, thus confirming the identity of this compound.

In conclusion, the non-targeted identification of the 7 veterinary drugs in honey as well as another compound beyond the list of spiked compounds was possible using All Ions MS/MS mode, showing the promising non-targeted applications of this method. Further studies are required to explore and optimize the characteristics of the non-targeted identification workflow using such type of data acquisition.

4. Conclusions

A fast screening and quantification method was successfully developed and validated for the targeted analysis of 7 veterinary drug residues in honey, using direct injection HPLC-QTOF-MS. This method allows for the detection of the selected veterinary drug residues at levels approximately 20–100 times lower than the actual regulatory limits, with acceptable recoveries, linearity and repeatability. The total analysis time is only 45 min per sample (sample preparation + analysis in ESI + mode). Negative ionization could be added in the future to allow for the analysis of other residues (e.g. chloramphenicol). The method was successfully applied to 35 honey samples from the Canadian market. Tylosin A, tylosin B, sulfamethazine and sulfadimethoxine were detected in some samples at levels below the regulatory limits for honey in Canada. All Ions MS/MS data was recorded at four different voltages, allowing for the confirmation of the identity of both targeted and non-targeted compounds (e.g. HMF and sugar-sulfonamides conjugates). The continuous recording of accurate mass and All Ions MS/MS data could also allow for non-targeted screenings of other compounds (e.g. pesticides), and this approach will be studied in future work.

Acknowledgments

We wish to acknowledge financial support from the Fonds de Recherche du Québec - Nature et Technologies (FRQ-NT NC-198270) and the Canada Foundation for Innovation/John R. Evans Leaders Fund grant (Project #35318) of S. Bayen. We also wish to thank Prof. V. Yaylayan for the synthesis of the sugar-sulfonamide conjugates.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfda.2018.12.013.

REFERENCES

[1] Klein AM, Vaissiere BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, et al. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. B-Biol. Sci. 2007;274:303–13.

[2] Steffan-Dewenter I, Potts SG, Packer L. Pollinator diversity and crop pollination services are at risk. Trends Ecol Evol 2005;20:651–2.
[3] Reybroeck W, Daeseleire E, De Brabander HF, Herman L. Antimicrobials in beekeeping. Vet Microbiol 2012;158:1–11.
[4] Health Canada. List of maximum residue limits (MRLs) for veterinary drugs in foods. 2017.
[5] Health Canada. Working residue levels (WRL) for antimicrobial residues in honey. 2005.
[6] Thompson TS, Noot DK, Calvert J, Pernal SF. Determination of lincomycin and tylosin residues in honey using solid-phase extraction and liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J Chromatogr A 2003;1020:241–50.
[7] Lopez MI, Pettis JS, Smith IB, Chu PS. Multiclass determination and confirmation of antibiotic residues in honey using LC-MS/MS. J Agric Food Chem 2008;56:1553–9.
[8] Orso D, Floriano L, Ribeiro LC, Bandeira NMG, Prestes OD, Zanella R. Simultaneous determination of multiclass pesticides and antibiotics in honey samples based on ultra-high performance liquid chromatography-tandem mass spectrometry. Food Anal. Methods. 2016;9:1638–53.
[9] Bayen S, Yi XZ, Segovia E, Zhou Z, Kelly BC. Analysis of selected antibiotics in surface freshwater and seawater using direct injection in liquid chromatography electrospray ionization tandem mass spectrometry. J Chromatogr A 2014;1338:38–43.
[10] Ciofi L, Renai L, Rossini D, Ancillotti C, Falai A, Fibbi D, et al. Applicability of the direct injection liquid chromatographic tandem mass spectrometric analytical approach to the sub-ng L-1 determination of perfluoro-alkyl acids in waste, surface, ground and drinking water samples. Talanta 2018;176:412–21.
[11] Bayen S, Estrada ES, Juhel G, Kelly BC. Direct injection of tissue extracts in liquid chromatography/tandem mass spectormetry for the determination of pharmaceuticals and other contaminants of emerging concern in mollusks. Anal Bioanal Chem 2015;407:5553–8.
[12] Olmo-García L, Bajoub A, Monasterio RP, Fernandez-Gutierrez A, Carrasco-Pancorbo A. Metabolic profiling approach to determine phenolic compounds of virgin olive oil by direct injection and liquid chromatography coupled to mass spectrometry. Food Chem 2017;231:374–85.
[13] Du B, Wu LM, Xue XF, Chen LZ, Li Y, Zhao J, et al. Rapid screening of multiclass syrup adulterants in honey by ultrahigh-performance liquid chromatography/quadrupole time of flight mass spectrometry. J Agric Food Chem 2015;63:6614–23.
[14] Mol HGJ, Plaza-Bolanos P, Zomer P, de Rijk TC, Stolker AAM, Mulder PPJ. Toward a generic extraction method for simultaneous determination of pesticides, mycotoxins, plant toxins, and veterinary drugs in feed and food matrixes. Anal Chem 2008;80:9450–9.
[15] Gomez-Perez ML, Plaza-Bolanos P, Romero-Gonzalez R, Martinez-Vidal JL, Garrido-Frenich A. Comprehensive qualitative and quantitative determination of pesticides and veterinary drugs in honey using liquid chromatography-Orbitrap high resolution mass spectrometry. J Chromatogr A 2012;1248:130–8.
[16] Kivrak I, Kivrak S, Harmandar M. Development of a rapid method for the determination of antibiotic residues in honey using UPLC-ESI-MS/MS. Food Sci Technol 2016;36:90–6.
[17] Cesen M, Lambropoulou D, Laimou-Geraniou M, Kosjek T, Blaznik U, Heath D, et al. Determination of bisphenols and related compounds in honey and their migration from selected food contact materials. J Agric Food Chem 2016;64:8866–75.
[18] Cicero N, Naccari C, Cammilleri G, Giangrosso G, Cicero A, Gervasi T, et al. Monitoring of neonicotinoid pesticides in beekeeping. Nat Prod Res 2017;31:1258–62.
[19] Martinello M, Borin A, Stella R, Bovo D, Biancotto G, Gallina A, et al. Development and validation of a QuEChERS method coupled to liquid chromatography and high resolution mass spectrometry to determine pyrrolizidine and tropane alkaloids in honey. Food Chem 2017;234:295–302.
[20] Gwinn MR, Axelrad DA, Bahadori T, Bussard D, Cascio WE, Deener K, et al. Chemical risk assessment: traditional vs public health perspectives. Am J Public Health 2017;107:1032–9.
[21] Fu YQ, Zhao CX, Lu X, Xu GW. Nontargeted screening of chemical contaminants and illegal additives in food based on liquid chromatography-high resolution mass spectrometry. Trac Trends Anal Chem 2017;96:89–98.
[22] Knolhoff AM, Zweigenbaum JA, Croley TR. Nontargeted screening of food matrices: development of a chemometric software strategy to identify unknowns in liquid chromatography-mass spectrometry data. Anal Chem 2016;88:3617–23.
[23] Perez-Ortega P, Lara-Ortega FJ, Garcia-Reyes JF, Gilbert-Lopez B, Trojanowicz M, Molina-Diaz A. A feasibility study of UHPLC-HRMS accurate-mass screening methods for multiclass testing of organic contaminants in food. Talanta 2016;160:704–12.
[24] van Asselt ED, van der Spiegel M, Noordam MY, Pikkemaat MG, van der Fels-Klerx HJ. Risk ranking of chemical hazards in food-A case study on antibiotics in The Netherlands. Food Res Int 2013;54:1636–42.
[25] Sheridan R, Policastro B, Thomas S, Rice D. Analysis and occurrence of 14 sulfonamide antibacterials and chloramphenicol in honey by solid-phase extraction followed by LC/MS/MS analysis. J Agric Food Chem 2008;56:3509–16.
[26] Pang GF, Cao YZ, Zhang JJ, Jia GQ, Fan CL, Li XM, et al. Simultaneous determination of 16 sulfonamides in honey by liquid chromatography/tandem mass spectrometry. J AOAC Int 2005;88:1304–11.
[27] Wang YH, Li L, Chen CH, Cheng SR, Lee MS, Yu PP, et al. Investigation of breaking efficiency of N-glycosidic bond between sulfonamide and honey. Chem Res Chin Univ 2012;28:195–9.
[28] Ranasinghe A, Ramanathan R, Jemal M, D'Arienzo CJ, Humphreys WG, Olah TV. Integrated quantitative and qualitative workflow for in vivo bioanalytical support in drug discovery using hybrid Q-TOF-MS. Bioanalysis 2012;4:511–28.
[29] Xia YQ, Lau J, Olah T, Jemal M. Targeted quantitative bioanalysis in plasma using liquid chromatography/high-resolution accurate mass spectrometry: an evaluation of global selectivity as a function of mass resolving power and extraction window, with comparison of centroid and profile modes. Rapid Commun Mass Spectrom 2011;25:2863–78.
[30] Croley TR, White KD, Callahan JH, Musser SM. The chromatographic role in high resolution mass spectrometry for non-targeted analysis. J Am Soc Mass Spectrom 2012;23:1569–78.
[31] Matuszewski BK, Constanzer ML, Chavez-Eng CM. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem 2003;75:3019–30.
[32] EU Reference Laboratories for Residues of Pesticides, Workflow to perform quantification by standard addition procedure, (Last updated July 2017).
[33] Grushka E, Grinberg N. Advances in chromatography. CRC Press; 2016.

[34] Kmellar B, Fodor P, Pareja L, Ferrer C, Martinez-Uroz MA, Valverde A, et al. Validation and uncertainty study of a comprehensive list of 160 pesticide residues in multi-class vegetables by liquid chromatography-tandem mass spectrometry. J Chromatogr A 2008;1215:37–50.
[35] Kasiotis KM, Anagnostopoulos C, Anastasiadou P, Machera K. Pesticide residues in honeybees, honey and bee pollen by LC-MS/MS screening: reported death incidents in honeybees. Sci Total Environ 2014;485:633–42.
[36] Australian Pesticides and Veterinary Medicines Authority. Agricultural and veterinary chemicals code instrument No. 4. MRL Standard; 2012.
[37] Commission Regulation (EU). No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Official Journal of the European Union; 2010. p. 1–72.
[38] U. S. Food and Drug Administration (FDA). Tolerances for residues of new animal drugs in food, Code of Federal Regulations. Title 1975;21.
[39] Mahmud I, Sternberg S, Williams M, Garrett TJ. Comparison of global metabolite extraction strategies for soybeans using UHPLC-HRMS. Anal Bioanal Chem 2017;409:6173–80.
[40] Galuszka A, Migaszewski Z, Namiesnik J. The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices. Trac Trends Anal Chem 2013;50:78–84.
[41] Canadian Food Inspection Agency (CFIA), reportNational chemical residue monitoring Program. 2013-2014 report, (2013-2014).
[42] Canadian Food Inspection Agency (CFIA), reportNational chemical residue monitoring Program. 2012-2013 report, (2012-2013).
[43] Kochansky J. Degradation of tylosin residues in honey. J Apicult Res 2004;43:65–8.
[44] Thompson TS, Pernal SF, Noot DK, Melathopoulos AP, van den Heever JP. Degradation of incurred tylosin to desmycosin - implications for residue analysis of honey. Anal Chim Acta 2007;586:304–11.
[45] Alippi AM, Albo GN, Leniz D, Rivera I, Zanelli ML, Roca AE. Comparative study of tylosin, erythromycin and oxytetracycline to control American foulbrood of honey bees. J Apicult Res 1999;38:149–58.
[46] Bohm DA, Stachel CS, Hackenberg R, Gowik P. Preparation and characterisation of in-house reference material of tylosin in honey and results of a proficiency test. Anal Chim Acta 2011;700:58–62.
[47] Joint FAO/WHO Expert Committee on Food Additives (JECFA). Evaluation of certain veterinary drug residues in food. Seventieth report of the joint FAO/WHO Expert committee on food Additives, WHO technical report series 954. 2008.
[48] Mitchell SM, Ullman JL, Teel AL, Watts RJ. Hydrolysis of amphenicol and macrolide antibiotics: chloramphenicol, florfenicol, spiramycin, and tylosin. Chemosphere 2015;134:504–11.
[49] Gerona RR, Schwartz JM, Pan J, Friesen MM, Lin T, Woodruff TJ. Suspect screening of maternal serum to identify new environmental chemical biomonitoring targets using liquid chromatography-quadrupole time-of-flight mass spectrometry. J Expo Sci Environ Epidemiol 2018;28:101–8.
[50] Wang J, Leung D. Analyses of macrolide antibiotic residues in eggs, raw milk, and honey using both ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry and high-performance liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2007;21:3213–22.
[51] Tornuk F, Karaman S, Ozturk I, Toker OS, Tastemur B, Sagdic O, et al. Quality characterization of artisanal and retail Turkish blossom honeys: determination of physicochemical, microbiological, bioactive properties and aroma profile. Ind Crop Prod 2013;46:124–31.