1. Introduction
A natural food as honey is subject of diverse restrictions for contaminants presence as the case of antibiotics including sulfonamides. It is worldwide practice by Beekeepers to use antibiotics as therapeutic agents to treat bee bacterial brood diseases, however the use of sulfonamides (SA) and trimethoprim (TMP) in beekeeping practice is prohibited in EU. There are no a worldwide MRLs for sulfonamides in honey in the EU, but a minimum required performance level (MRPL) was set for analytical methods at a level of 10 µg/kg of sulfonamides, encouraging the development of reliable laboratory methods for detection in diverse foods included honey. SAs and TMP poses threats to public health causing allergic reactions and microorganisms’s drug resistance. Considering MRLs and MRPL of SAs and TMP in food products from animals tend to be continually reduced to preserve human health safety, HPLC-MS/MS is an effective strategy to characterize and accurately measure those antibiotics. The present method is selective, fast, and very sensitive for 20 sulfonamides and trimethoprim.

2. Materials and Method
Sample preparation
5 grams of honey were acidified with 5 mL 2.0M HCl vortexed 1 min and sonicated 15 minutes before to spike with standard mixture of 21 sulfonamides and trimethoprim at 10 ppb and 50 ppb. Extraction were performed using QuEChERS method with extraction salts and diverse clean-up sorbents following manufacturer’s procedure (Supelco Supel™ QuE Citrate, PSA/C18, Zexp/C18, Zexp and Zexp+) with a final 1.5 extraction dilution using methanol to inject in the LC/MS system. A multiple reaction monitoring MRM method was optimized for quantitation for each sulfonamide compound using a Shimadzu Nexera UHPLC with an LCMS-8060 fast-scanning triple quadrupole mass spectrometer model equipped with software LabSolution LCMS version 5.65 and electrospray ionization ESI.

Stock standard solutions of each compound were prepared by dissolving weighed amounts in methanol alkaline (3M with NaHCO3) then diluting to 100 µg/mL with mobile phase A:B 50:50 and a final dilution to 1000 ng/mL using matrix extracts from honey without SAs and TMP spiked. Table 1 shows the concentrations at each 17 levels used to build calibration curves for external validation method. For every level each was prepared using matrix matched extracts from honey without SAs and TMP spiked with each QuEChERS SPE cleanup sorbent. (Figure 2).

LC conditions
A Kromasil 24u PFP 100 A column (100 × 2.1 mm) was used at 30°C, flow rate of 0.5 mL/min, and 5 µL injection volume. A binary gradient of 10% methanol, 3.9% formic acid (mobile phase A) and methanol, 0.3% formic acid (mobile phase B) was used with the gradient program described in Table 1.

Table 2. HPLC gradient used

Mass Spectrometry
ESI was used in positive mode, spray voltage was 4.5 kV, desolvation line temperature was 250°C, nebulization gas was 3.0 L/min, heating gas flow 10L/min, interface temperature was 300°C, heater block was 400°C, and drying gas 10 L/min.

To implement sulfonamide quantitation, MRM transitions were optimized using a 0.5 µg mixture of SAs, 1µL injections at 400µL/min. Three transitions from parent ions and fragments were selected using the optimization tool software.

3. Results

Figure 2: Average (n=3) recovery of honey spiked with 10µg/Kg (Figure 2A) and 50µg/Kg (Figure 2B) using diverse SPE sorbents. Authentic SAs standards were fully characterized by HPLC and MS/MS with an MRM optimized assay. Figure 2 shows better performance for QuEChERS extractions with SPE sorbent PSA/C18 instead of QuEChERS quantification LOQ as low as 0.015 ppb (15pp) for some SAs and trimethoprim using calibration curves diluted with HPLC buffers and as low as 0.03 ppb (30pp) when calibrators were dissolved in matrix extracted from commercial organic honey free of drugs diluted 1:5 in MeOH. The recovery of SAs compounds and trimethoprim (Figure 2) spiked on that honey reported a range of 85 to 122% for 10µg/kg of spiked compounds and a range of 99 to 122% when spiked with 50µg/kg. Only sulfanilamide presented a poor recovery, reporting 10% when 100µg/kg was spiked but an acceptable recovery of 58% when 50 µg/kg was spiked, suggesting a matrix effect of honey causing its considered and also an easy loss by the sulfanilamide double amino group strong interaction with the primary secondary amino PSA sorbent. LOQs are described in Figure 1. Calibration curves of standards in matrix extracted from honey were linear with r2 > 0.990 in the tested range as shown in Figure 3.

4 Conclusions
LC-MS/MS with appropriate selection of QuEChERS SPE sorbent as extraction method provides a fast, simple, accurate and high sensitive method for analysis of sulfonamides drugs and trimethoprim in honey with also excellent recovery range improved from previous studies with matrix matched calibration.

For Research Use Only. Not for use in diagnostic procedures.