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Research Article

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Development of An LC-MS Method for The Determination and Identification of Sulfonamide Residues in Propolis

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ABSTRACT

A LC-MS method was developed in order quantify any contamination by five sulfonamides including sulfamethazine SMZ, sulfathiazole STZ, sulfadioxine SDX, sulfadimethoxine SDMX, and sulfamethoxazole SMXZ in propolis samples. The method showed satisfactory results based on the calibration data where the R^2 was > 0.9802 for all the sulfonamides and LOD and LOQ was within the range 24.26 ng/ml to 31.89 ng/ml and 34.00 ng/ml to 49.63 ng/ml for analysis by tandem mass using a TSQ 7000 instrument. In contrast use of an LTQ Orpitrap instrument gave lower limits of 0.79 ng/ml to 4.39 ng/ml and 2.49 ng/ml to 7.02 ng/ml as for the LOD and LOQ respectively. The methods showed no sign of sulfonamides residues in any of the propolis samples examined above the LOD. The LTQ Orbitrap method is better suited for applications requiring ultra-low detection limits, while the TSQ remains practical for less demanding analytical scenarios.

Keywords- *Propolis; Liquid Chromatography; Sulphonamides.*

INTRODUCTION

Propolis, commonly known as bee glue, exhibits a diverse range of colors, including cream, yellow, green, and shades of brown from light to dark. Its texture also varies significantly: some samples are friable and hard, while others display an elastic, gummy consistency.[1] Propolis, a resinous compound gathered by bees from leaf buds and plant exudates (such as resins, gums, latexes, mucilage, and lipophilic substances on leaves or wounded plant tissues), is synthesized by worker bees. During collection, they blend these plant-derived materials with beeswax and the enzyme β-glucosidase. The resulting mixture serves dual purposes: sealing hive cracks to insulate against drafts and fortifying the colony against pathogens or invaders.[2, 3]

Propolis has garnered significant scientific interest due to its wide range of pharmacological and biological properties. Studies highlight its antimicrobial, anti-inflammatory, antifungal, antiviral, anticarcinogenic, immunomodulating[4], and antioxidant effects, positioning it as a versatile natural product with therapeutic potential.[5]

Researchers have reported many different compound groups as the main constituents of propolis, such as; flavonoids (flavones, flavonols, flavanones, dihydroflavonols and chalcones); phenolic acids; phenolic aldehydes; polyphenolic derivatives (cinnamic and benzoic acid, caffeic acid esters and terpenes)[6] and other compounds such as, wax 30%, essential oils10%, pollen 5% and various organic compound 5%.[1]

Various techniques have been reported in the literature for the analysis of propolis. The use of chromatographic techniques such as thin layer chromatography (TLC), gas chromatography (GC), high performance liquid chromatography (HPLC), coupled to mass spectrometry[7] have been reported for analysis and identification of compounds in some types of propolis including flavonoids such as pinobanksin, quercetin, naringenin, galangine, chrysin and cafeic acid.[8]

Bee products are susceptible to contamination by various xenobiotics, with environmental factors and beekeeping practices serving as primary contributors. Pollutants such as heavy metals (lead, cadmium, mercury), radioactive isotopes, persistent organic pollutants, and pesticides present in the hive's surrounding environment can infiltrate bees and subsequently taint honey, propolis, and other hive-derived material.[9,10] These contaminants often originate from industrial emissions, agricultural runoff, or improper hive management, posing risks to both bee health and product safety.[11]

The use of antibiotics in beekeeping, particularly for treating bacterial infections like American and European foulbrood diseases caused by Paenibacillus larvae and Melissococcus plutonius, respectively, has been identified as a key contributor to contamination in bee products.12 Tetracycline, streptomycin, sulfonamides, and chloramphenicol are among the most frequently reported antibiotics employed by beekeepers.[9] Notably, some of these compounds such as oxytetracycline[12], streptomycin, and sulphonamides13 are also applied in agriculture to combat fruit tree diseases like fire blight.[12] When blossoms absorb high concentrations of these antibiotics, bees foraging on treated plants may inadvertently transfer residues into bee products, elevating contamination risks.[10]

Sulfonamides, a class of antimicrobial agents, are extensively utilized in veterinary medicine to treat a broad spectrum of bacterial and protozoan infections in livestock such as cattle, swine, and poultry, targeting diseases like mastitis and coccidiosis.10 Beyond therapeutic applications, they are also routinely incorporated into animal feed as growth promoters. For instance, sulfathiazole and other sulfonamides compounds are documented for its use in treating honeybee colonies, particularly against bacterial

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pathogens.[14]

In recent years, emerging research has underscored the growing risk of antibiotic contamination particularly sulfonamides residue in honey and other bee products, raising significant concerns for food safety and public health. These residues pose dual threats: compromising product quality and contributing to antibiotic resistance in both bee microbiomes and human gut flora.[14]

MALERIALS AND METHODS

Chemicals and Reagents

The reference standards sulfamethazine (SMZ), sulfathiazole (STZ), sulfadioxine (SDX), sulfadimethazine (SDMZ), and sulfamethoxazole (SMXZ), and were purchased from Sigma Aldrich, Dorset U.K. HPLC grade formic acid, acetonitrile, methanol and water were purchased from Fisher Scientific U.K. The propolis samples were provided by Natures Laboratory Ltd U.K. The propolis samples were stored at room temperature.

Standard Preparation

Stock solutions (1mg/ml) of the individual standards of sulfonamides were prepared by dissolving 100 mg of the substance in methanol then making it up to 100 ml with methanol in a volumetric flask. The 1mg/ml standard solutions of sulfonamides were used to prepare an intermediate standard stock solution of 10 μg/ml by transferring 1ml from each individual sulfonamide standard (1mg/ml) solution into a 100 ml volumetric flask and making it up with methanol to 100ml.

Preparation of Calibration Solutions

A quantitative study was carried out by using STZ, SMZ, SDX, SMXZ, and SDMX solutions. Varying volumes of intermediate stock solution was diluted to prepare a series of standard solutions which were used to construct a calibration curve within a limited range of concentration 0.05 - 0.125 μg/ml.

Spiked Propolis Solutions

An extract was prepared by weighing 50mg of propolis raw material and then transferring it into a 50 ml volumetric flask and 25 ml methanol was added to dissolve the propolis and the volume was completed with 0.1% v/v formic acid to have final propolis solution (1mg/ml) and the same procedure repeated for each time propolis solution need. The propolis solution was spiked with the standard solution in range of 0.05- 0.125 μg/ml and filtered with a nylon 0.2μm syringe filter before injecting into the High performance liquid chromatography (HPLC) system.

Instrumentation

High performance liquid chromatography (HPLC) analyses were carried out on a Thermo Separation® Product (TSP) system (San Jose, CA, USA) using of a TSP P4000 pump, connected to a TSP AS3000 auto sampler, and a TSP UV6000 PDA UV- detector. The experiment was carried out using a reverse phase system with a H5ODS column (Hypersil ODS 150mm x 4.6 mm) from Hichrom Ltd Reading U.K. The column was fitted with a C18 security guard column.

The mobile phase consisted of 0.1 % v/v formic acid prepared by transferring 1ml of HPLC grade formic acid to a 1000 ml volumetric flask then making up to 1000ml with HPLC grade water.

A mobile phase with flow rate of 0.4 ml min^{-1} at 25 ºC was delivered through the pump in isocratic mode. The best separation was achieved by using 75 % of formic acid (0.1 % v/v) as aqueous phase and 25 % of acetonitrile as the organic phase for 30 min and then switching to 40% of aqueous and 60 % of organic in order to clean the column of the waxy propolis constituents which are highly hydrophobic.

Tandem Mass Spectrometry Instrument

The HPLC system was coupled with a Finnigan MAT triple stage quadrupole (TSQ) 7000 mass spectrometer (TSP, San Jose, USA) and electrospray ionization (ESI) was used for the detection of the analytes. The ion source and the capillary temperature were set to 250 ^{O}C, polarity was positive mode and the LC method and the mass spectrometry parameters were setup using the *x*-calibur® software. Nitrogen was used as the sheath and auxiliary gas (60 bar), and the needle voltage was maintained at 4.5 kV for all analytes. The collision gas argon was set at 1 Torr and the CID energy was set to 30 v for fragmentations in MS/MS mode.

LTQ Orbitrap Instrument

A Finnigan™ LTQ™ Linear ion trap instrument (Thermo Electron Corporation, San Jose, Ca, USA) coupled with a Fourier transform LTQ Orbitrap™ (Thermo Electron Corporation, San Jose, Ca, USA) was used for identifying the sulfonamides residue in propolis extracts. The system was coupled with Surveyor HPLC system (Thermo Electron Corporation, San Jose, Ca, USA) which was composed of a Surveyor MS pump, Surveyor AS autosampler. *x*-calibur® software version 2.0 (Thermo Electron Corporation, San Jose, Ca, USA) was used for the acquisition of data. The ion source and the capillary temperature were set to 250 ^{O}C, polarity was positive mode and ESI voltage 4.5 kv the LC method and the mass parameter were setup using the *X-calibur*® software. Nitrogen was used as the sheath and auxilliary gas.

The HPLC conditions used were the same as those used with the TSQ instrument.

RESULTS

An experiment was carried out using a standard solution containing the five sulfonamides (STZ, SMZ, SDX, SMXZ, SDMZ) which were injected into the HPLC was carried out using isocratic mode of the mobile phase delivery as 75% of 0.1 % v/v formic acid and 25 % acetonitrile for separation on a H5ODS column. Since the LC separation of sulfonamides depends on the polarity and ionization of the sulfonamide and the pH of mobile phase was considered as important factor for the analytes separation. LC MS is incompatible with many mobile

phases, and it was decided to use formic acid with acetonitrile as organic modifier. The use of a high percentage of aqueous phase is required due to the polarity of sulfonamides and using the formic acid gave an adequate separation as shown in (Figure 1). Under the optimum HPLC conditions the standard solution containing five sulfonamide compounds was injected and the separation monitored using the full-scan mode generating, the total ion current (TIC) and UV chromatograms (280 nm). Separation was achieved in 30 min without derivatization and the peaks were both sharp and well resolved.

The quadrupole was set up to scan the analytes over the range *m/z* 100-500. It was observed that all the sulfonamide standard mass spectra at the optimum needle voltage (4.5 V) gave the expected protonated molecular ions $[M+H]^+$ for each compound sulfamethoxazole (SMXZ *m/z* = 254), sulfadimethoxine (SDMX *m/z* 311), sulfamethazine (SMZ *m/z* 279), sulfadoxine (SDX *m/z* 311) and sulfathiazole (STZ *m/z* = 256). The aim at this stage was to confirm the $[M+H]^{+}$ ions for each analyte. That figure -2 shows the extracted ion chromatograms for the sulfonamides in the mixture.

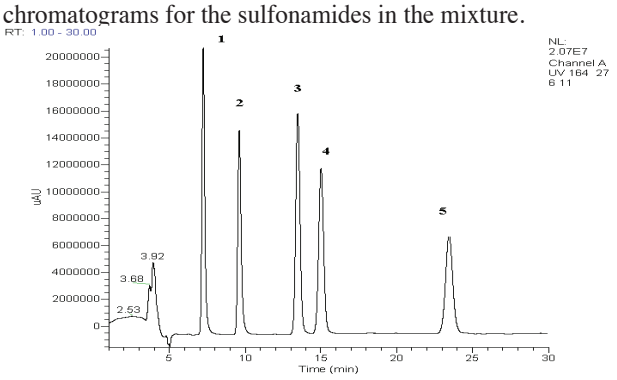


Figure 1: UV chromatogram (280nm) of injected sulfonamides solution at 10 μg/ml at 0.4ml/min using formic acid and acetonitrile (75:25 of 0.1% v/v) in as an isocratic mobile phase.

The peaks 1,2,3,4,5 represent STZ, SMZ, SDX, SMZX, and SDMX respectively.

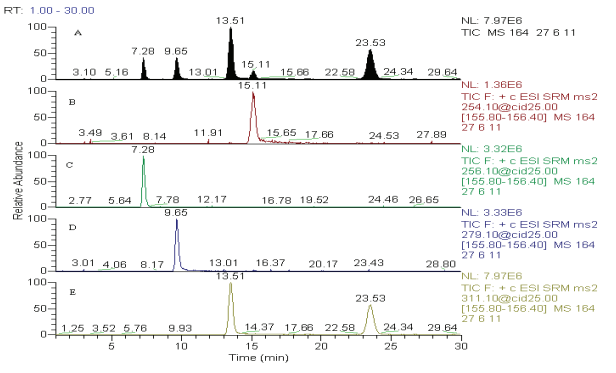


Figure 2: Total and extracted - ion chromatogram of the sulfonamides of 10μg/ml at 0.4ml/min using 75:25 of formic acid and acetonitrile in isocratic mobile phase mode.

Chromatogram A shows all of the extracted ions for the sulfonamides, and chromatogram B corresponded to SMZX, C corresponded to STZ, D corresponded to SMZ, and E represent SDX and SMZX as they have same molecular ion with different retention time.

The fragmentation on the sulfonamides was obtained by collision-induced dissociation (CID) and the fragmentation pattern was provided by mass spectra of the protonated molecules of each sulfonamide.

- SO 108 m/z

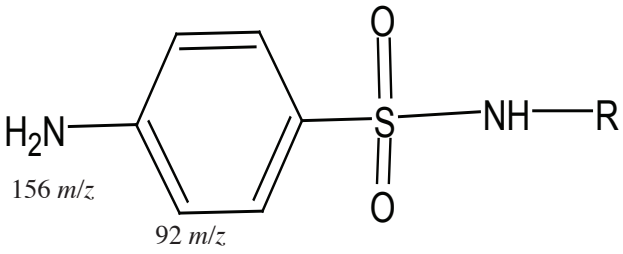
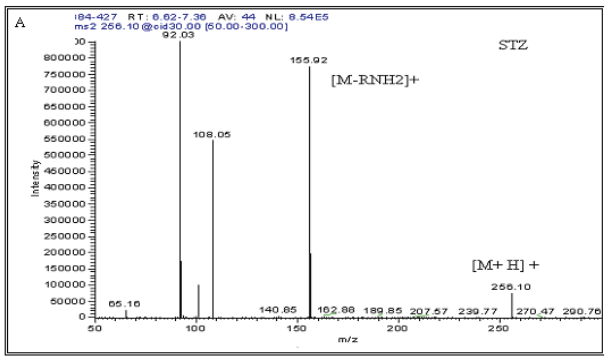


Figure 3: Scheme showing product ion formation for the sulfonamides following TSQ MS/MS fragmentation.

The ESI-MS/MS method was optimized to identify sulphonamide compounds by selecting protonated molecular ions ([M+H]) as precursor ions and monitoring characteristic product/fragment ions. As illustrated in figure 3, the fragmentation pattern typically generated ions at m/z 92, 108, and 156 for most analytes. The m/z 92 ion, corresponding to $[M - RNH^2 - SO]^+$, and the m/z 108 ion, attributed to $[M - RNH^2 - SO]^+$, were observed across all sulfonamides but exhibited weak signal intensities. The m/z 156 ion, arising from $[M - RNH^2]^+$, was detected only in a subset of compounds, further highlighting variability in fragmentation behaviour as shown in figure 4.



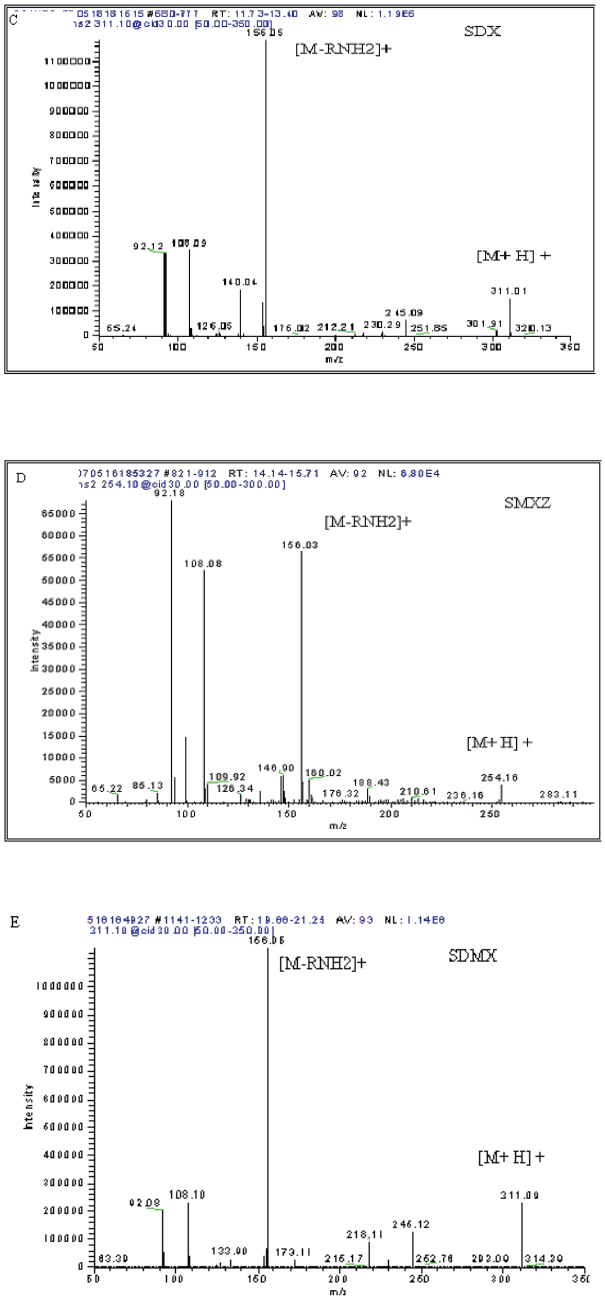


Figure 4: The ESI MS/MS full spectra show the identified protonated molecular ions [M+ H] $^{+}$ and product ions of sulfonamides; (A) STZ, (B) SMZ, (C) SDX, (D) SMXZ, and (E) SDMX respectively.

Although the molecular mass of SDX and SMDX was the same at *m/z* 311 and the product ion spectra contained the same ions, the retention times differed significantly from each other. In addition, a product ion at *m/z* 140 was found in the spectrum of SDX and was not observed in the spectra of the SMDX. Products ion at 124 m/z, 186 m/z of SMZ were observed in spectra, which are corresponding to [RNH2 + 2H] $^{+}$and [RNH_2+SO_2] $^{+}$ fragments.

Table -1: The precursor and product ions for the sulfonamides studied.

Analysts	Precursor Ion *(m/z)*	Product ions *(m/z)*	Retention time *(min)*
STZ	256 [M+ H] $^{+}$	156	7.18
SMZ	279 [M+ H] $^{+}$	156	9.95
SDX	311 [M+ H] $^{+}$	156	14.77
SMXZ	254 [M+ H] $^{+}$	156	13.32
SDMX	311 [M+ H] $^{+}$	156	23.09

A blank sample of propolis solution was analysed with same condition showed for sulfonamide standard calibration and the result demonstrate there was no possible inference between propolis constituents and sulfonamide. The calibration curve for sulfonamides in propolis was carried out by spiking a propolis solution with the five sulfonamide standards in the range of 0.05- 0.125 μg/ml. The reason for of using propolis solution for spiking was in order to see how the method coped with the sample background. Usually the quantification of drug residues is performed by using a matrix-matched calibration curve made from spiking blank samples using the same matrix as the real samples.

The calibration graphs were drawn by Excel software and gave acceptable linearity for the five sulfonamide standards in the blank propolis matrix in range from the 0.05 and up to 0.125 μg/ml. The linearity of the method was evaluated by calculation of the regression line which expressed by the correlation coefficients (R^2 value) which were all above 0.9803. Table 2 below summarises the calibration data for the sulfonamides.

Table 2: Summary of calibration data for STZ, SMZ, SDX, SMXZ, and SDMZ. Calibration curves obtained using LC-ESI-MS and the SRM mode.

Analysts	R^2 (mean± S.D, n= 3)	RSD%	Linear range (ng/ml)
STZ	0.9803 ± 0.004	0.437	50 - 125
SMZ	0.9926 ± 0.0057	0.57	50 - 125
SDX	0.9897 ± 0.0016	0.17	50 - 125
SMXZ	0.9954 ± 0.0012	0.13	50 - 125
SDMX	0.989 ± 0.0083	0.84	50 - 125

*RSD% Relative Standard Deviation

The sensitivity of the method was evaluated by determining the limit of detection (LOD) and quantification (LOQ) of the target analyts. The limit of detection was defined as the lowest concentration producing a chromatographic peak with a signal-to-noise ratio > 3/1 and lowest value obtained was 24.26ng/ml represented by the concentration of SDMX (Table 3).

The limit of quantification was defined as the lowest concentration it giving a peak height with a signal-to-noise ratio of 1:10 and for its confirmation SRM transition giving the ion *m/z* 156 was used for all the spiked propolis samples and lowest value was 34.00 ng /ml represented as the concentration of SDMX. The limit of detection and quantification was lower when the LTQ

instrument was used. That the lowest limits of detection were 1.65 ng/ml,1.14 ng/ml, 4.39 ng/ml, 0.93 ng/ml, 0.79 ng/ml for STZ, SMZ, SDX, SMXZ, and SDMX respectively (Table 3).

Table 3: LOD for STZ, SMZ, SDX, SMXZ, and SDMX using TSQ and LTQ with SRM mode

Analysts	TSQ		LTQ	
	Conc (ng/ml)	RSD % (n=3)	Conc (ng/ml)	RSD % (n=3)
STZ	26.20	4.19	1.65	0.49
SMZ	28.92	2.53	1.14	0.75
SDX	31.89	3.88	4.39	0.56
SMXZ	28.53	2.06	0,93	0.22
SDMX	24.26	4.02	0.79	0.37

This experiment demonstrates that the LOQ of the sulfonamides in spiked propolis samples was within 34.00 - 49.63 ng/ml using TSQ method. The lowest limits of quantification were 7.0 ng/ml, 2.49 ng/ml, 7.02 ng/ml, 3.73 ng/ml, and 4.57 ng/ml for STZ, SMZ, SDX, SMXZ, and SDMX respectively by using LTQ (Tables 4).

Table 4: The LOQ for STZ, SMZ, SDX, SMXZ, and SDMX using TSQ and LTQ with SRM mode.

Analysts	TSQ		LTQ	
	Conc (ng/ml)	RSD % (n=3)	Conc (ng/ml)	RSD % (n=3)
STZ	40.64	8.20	7	6.8
SMZ	43.00	4.03	2.49	4.38
SDX	49.63	1.28	7.02	2.1
SMXZ	40.07	4.83	3.73	0.6
SDMX	34.00	2.94	4.57	3.5

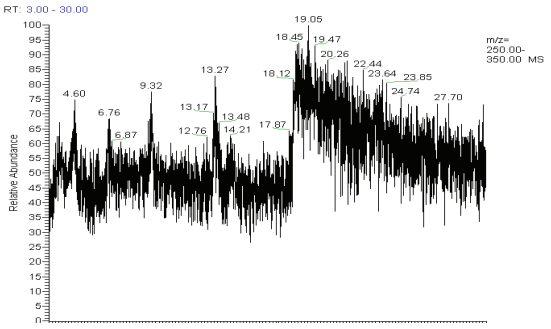
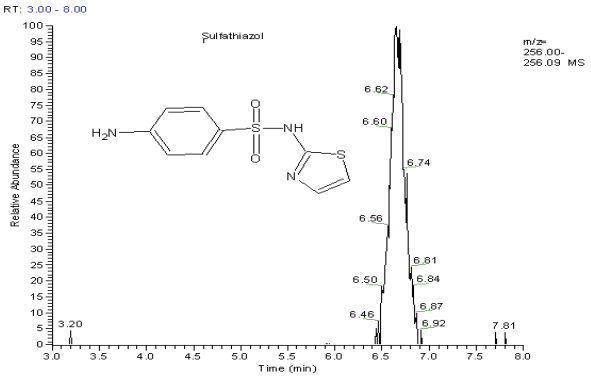


Figure 5: Total ion current 1mg/ml of propolis sample spiked with 50ng of STZ, SMZ, SDX, SMXZ, and SDMX using LTQ Orbitrap

instrument with zoom in mode using mass range 250-350 *m/z*.

A



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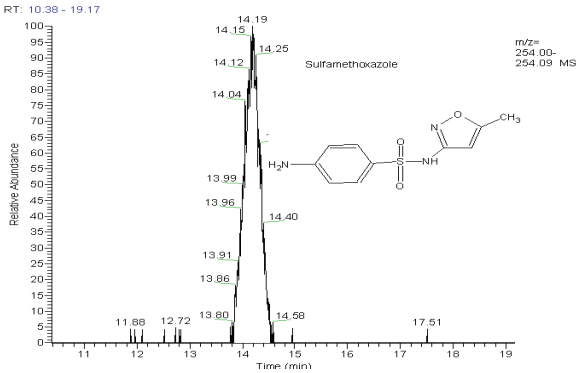

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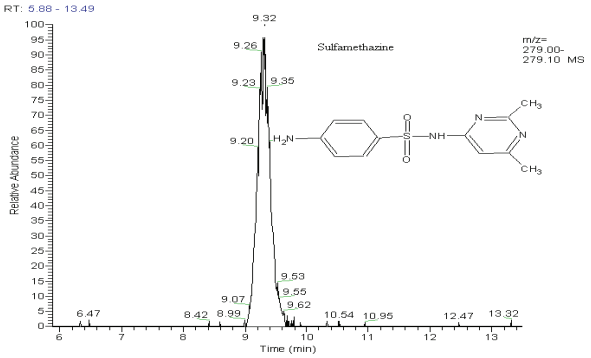

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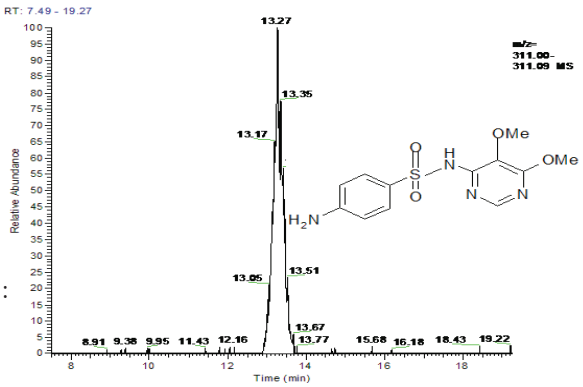

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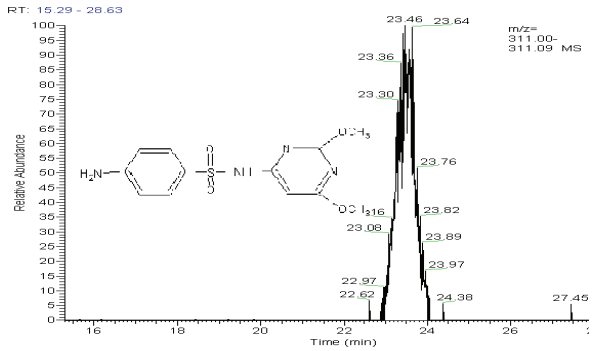

Figure 6: Sulfonamides in a 1 mg/ml of a propolis spiked sample with 50 ng of sulfonamides using LTQ Orbitrap spectrometer with restricted mass range for each compound, (A) STZ, (B) SMXZ, (C) SMZ, (D) SDX and (E) SDMX respectively.

A blank sample of propolis in a formic acid/ methanol solution was analysed with the same conditions used in the method by using LTQ Orbitrap and the results exhibit peaks at retention times (28.90, 25.39, and 15.34 min) within mass range *m/z* 250-350 (Figure 7). However, these peaks had masses *m/z* 287, 209, and 195 none of them matched the *m/z* of the sulphonamides. The explanation of these existing peaks in the chromatogram, that they may be attributed to the constituents of the propolis as none of them had the same mass as the sulfonamides.

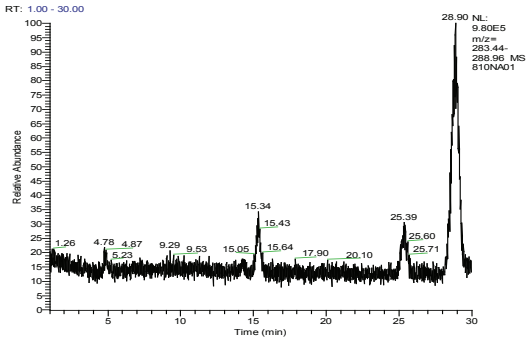

Figure 7: Chromatogram exhibiting different peaks resulting from analysed a blank propolis sample using the LTQ Orbitrap with mass a range 250-350 m/z with method same conditions.

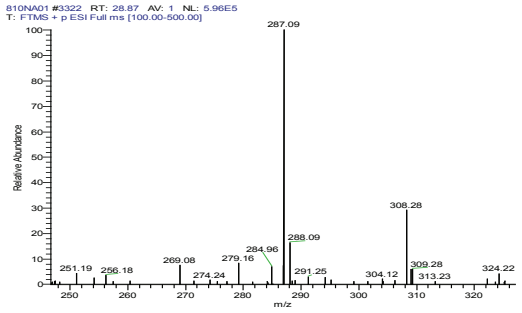

Figure 8: Mass spectrum showing a specific molecular ion at m/z 287.09 which can be attributed to the original constituent of the blank propolis sample.

The LTQ Orbitrap has identify peak which has molecular ion at m/z 287.09 as having a chemical formula $C_{15}H_{11}O_6$ which corresponds to kaempferol (MW 286.24) and a chemical formula of $C_{15}H_{10}O_6$.[7]

The analysis of blank propolis samples (unspiked with sulfonamides) revealed no detectable sulfonamide contamination, suggesting either their absence or concentrations below the method's limit of detection (LOD). The superior sensitivity of the LTQ Orbitrap compared to the TSQ instrument was evident in its enhanced mass accuracy, resolution, and lower LOD values. To further optimize the method's performance, restricting the monitored mass range to ±0.045 atomic mass units (amu) around the exact mass of each sulfonamide (Figure 5) significantly improved sensitivity and selectivity by minimizing background interference.

Figure 6 demonstrates that, extracting data within this narrow mass window further amplified detection precision, confirming that such targeted mass range adjustments enhance the method's robustness. The combination of the LTQ Orbitrap's advanced analytical capabilities and refined data acquisition strategies ensures reliable identification and quantification of sulfonamides, even at trace levels in complex matrices like propolis.

DISSCUSION

There are many studies cited in the literature for investigating of honey samples from different regions of the world and the researchers have trying to set up limit for sulfonamides residues

as a part of the public health concern especially after some research indicated the possibility of sulfonamides producing cancer.[13,15,16] Initially, the European Union, has setup allowable limits of sulfonamides in honey, which is below the limit of quantification (LOQ) of many of the routine analytical methods used in quality control laboratories. Analysis using chromatographic methods was agreed to be the only way analysis for chemical residues in food products (European Commission 2002/65/CE).[14,17] The sulfonamides the maximum residue limit MRL has been fixed in honey in some European countries between 10ng/g to 50ng/g.

In contrast there is no limit for sulphonamide residues cited for propolis which is one of the bee products introduced into food, health, and cosmetic products which mean the public can get the sulfonamides antibiotic from this additional source[17]tetracyclines, sulphonamides, β -lactams and chloramphenicol. Streptomycins, tetracyclines, sulphonamides (whole group. However, given that quantity of propolis consumed is much lower than the amount of honey that the limit need not be as low as that set for honey.

The sulfonamides differ only in the heterocyclic base attached to a sulfonamide moiety, which results in similar fragmentation for all the drugs. Since all the sulfonamides displayed weak signals for the ion at *m/z* 92 matching $[M\text{-}RNH_2\text{-}SO_2]^+$, and ion at m/z 108 $[M\text{-}RNH2\text{-}SO]^+$, these ions were not considered in this study for detecting and quantifying sulphonamides; However, the selection of the

156 m/z ion corresponding to $[M - RNH^2]^+$ for detecting sulfonamides in multiple reaction monitoring (MRM) mode is a common strategy in LC-MS/MS (Liquid Chromatography-Tandem Mass Spectrometry) analysis; because when sulfonamides subjected to collision-induced dissociation (CID), they often lose the amine group (RNH^2) from the sulfonamide moiety, generating a characteristic 156 m/z fragment (for unsubstituted sulfanilamide derivatives).[14,18] Additionally; pairing the 156 m/z fragment with a unique precursor ion for each sulfonamide, can distinguish between different sulfonamides in a mixture.[19] Moreover, a product ion at *m/z* 140 was found in the spectrum of SDX and was not observed in the spectra of the SMDX.; while products ion at 124 m/z, 186 m/z of SMZ were observed in spectra, which are corresponding to $[RNH2 + 2H]^+$ and $[RNH_2+SO_2]^+$ fragments. The ion of 204 m/z matching to $[RNH_2+SO_2+H_2O]^+$ was not observed in the SMZ spectra (Figure-4 B), but it has been reported in the literature by using ion trap MS-M.[20,21]

Since the *m/z* 156 fragment ion was shared between all the sulfonamides and the other fragment ions varied for each compound and could be attributed to the variation in the R group.[18,22] The m/z 156 ion was used for quantification of the sulfonamides and determination of the LOD and LOQ.

The experiment revealed that the limits of detection (LOD) and quantification (LOQ) for sulfonamides in spiked propolis samples varied between the TSQ and LTQ instruments. For the TSQ system, the LOD ranged from 24.26 to 31.89 ng/ml, while the LOQ was notably higher, spanning 34.00 to 49.63 ng/ml. In contrast, the LTQ instrument demonstrated significantly lower sensitivity thresholds, with an LOD range of 0.79–4.39 ng/ml and an LOQ range of 2.49–7.02 ng/ml. Among the tested sulfonamides, SDMX consistently exhibited the lowest detection and quantification limits, whereas SDX showed the highest values across both methods.

These results highlight the superior performance of the LTQ system in achieving lower detection and quantification limits compared to the TSQ, underscoring its enhanced sensitivity for analyzing sulfonamides in complex matrices like propolis. Given that the maximum permissible limit for sulfonamides in honey is established between 10–50 ng/g and considering that propolis is consumed in significantly smaller quantities compared to honey, the detection and quantification limits achieved by the current method (particularly using the LTQ system) appear adequate for regulatory compliance. The LTQ-derived LOQ range of 2.49–7.02 ng/ml falls well below the lower threshold of the regulatory limit (10 ng/g), ensuring sufficient sensitivity to detect sulfonamide residues at levels far stricter than the established safety standards. While the TSQ system showed higher LOD/LOQ ranges (24.26–31.89 ng/ml and 34.00–49.63 ng/ml, respectively), these values still align closely with the upper bounds of the regulatory range for honey. Combined with the lower dietary intake of propolis, the method's performance especially with the LTQ instrument demonstrates practical suitability for monitoring sulfonamide residues in propolis, offering a robust margin of safety for consumer protection.

The analysis of a blank propolis sample under the same conditions as the method revealed peak within the m/z 250–350 range (Figure 8), with notable mass at m/z 287 peak does not matched the m/z values of the target sulfonamides, suggesting they likely originated from natural propolis constituents. Further investigation using the LTQ Orbitrap identified the peak at m/z 287.09 as corresponding to a molecular formula of $C_{15}H_{11}O_6$ consistent with kaempferol (theoretical molecular weight 286.24 g/mol corresponding to $C_{15}H_{11}O_6$)[8], a known flavonoid in propolis. Crucially, no sulfonamide contamination was detected in the blank sample, indicating either their absence or concentrations below the method's LOD. This observation aligns with the superior sensitivity of the LTQ Orbitrap compared to the TSQ, as evidenced by its lower LOD/LOQ values, enhanced mass accuracy, and higher resolution. These attributes enabled full-scan analysis across a broad m/z 100–500 range while maintaining precision. To further optimize the method's sensitivity and selectivity, restricting the monitored mass range to ±0.045 amu around the exact mass of each sulfonamide (Figure 6) significantly reduced background noise, improving detection limits. The combination of the LTQ Orbitrap's advanced analytical capabilities and the refined mass range extraction underscores the method's robustness for detecting trace sulfonamides in complex matrices like propolis, even at levels far below regulatory thresholds.

So far, the maximum residue limitation (MRL) of sulfonamides has not been set in propolis products, however Europe, Canada and USA established action for any edible tissue product which states that a sulfonamide concentration not exceed 100μg/kg (Food and Drug Regulation 1991, EU Regulation).[23] While propolis is widely consumed for its health benefits, its regulatory status remains ambiguous.

Residue limits from honey or other bee products are sometimes extrapolated, but specific MRLs for propolis are urgently needed to ensure safety and align with international trade standards.

CONCLUSION

This study developed an LC-MS method for quantifying five sulfonamide residues in propolis across a concentration range of 0.05–0.125 µg/mL, validated for sensitivity, selectivity, linearity, and precision. The method achieved acceptable limits of detection (LOD) and quantification (LOQ) using both TSQ and LTQ Orbitrap instruments, demonstrating suitability for regulatory compliance

The LTQ Orbitrap's high mass accuracy and resolution enhance confidence in identifying low-abundance sulfonamides. Current performance aligns with residue limits for honey (a comparable matrix), but extraction refinements could bridge sensitivity gaps for propolis.

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