Review of Literature-

Literature review for the present study was done by referring various national and international Journals, published articles in different official standard books and referred Journals.

Jin et al (2017) developed an analytical method for the analysis of a wide range of veterinary drugs in honey. Modified sample extraction procedures were developed, followed by liquid chromatography tandem mass spectrometry determination injection. Use of the single sample preparation method for analysis of 42 veterinary drugs becomes more valuable because honey belong to completely different complex matrices and higher sensitivity than reference methods of China.

Ciulu et al (2016) stated that, honey is one of the most renowned natural foods. In particular, great attention has been paid by the scientific community towards classes of antibiotic compounds like streptomycin, sulphonamides, due to their capability to act as bacteriostatic. Moreover, the validation procedures were developed to estimate various antibiotics using liquid chromatography. Finally, results concerning the evaluation of the antibiotic residues were estimated and the validation of a new analytical approaches were reviewed.

Kivrak I et al (2016) validated a method for the simultaneous analysis of antibiotics, belonging to different classes sulfonamides, tetracycline, in honey samples. The method developed consists of ultrasonic extraction followed by LC–MS/MS with electrospray ionization in both positive mode and negative mode. Finally, the method developed was applied to the determination of target analytes in honey samples. The uniqueness of this study lies in the simultaneous determination of a remarkable number of compounds belonging to 23 drug at the sub-nanogram per kilogram level.

Guidi et al (2015) had developed a method for the estimation of chloramphenicol using LC-MS. It has been banned from food producing animals because of serious adverse effects to human health. However, it is still being used in some countries because of its high efficacy and relatively low price. There is currently a minimally required performance limit (MRPL) defined at 0.3 mg/kg. This is the reason why chloramphenicol has often been analyzed by highly efficient and sensitive single residue methods.

Sichilongo et al (2015) developed a very sensitive, simple and cost-effective liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS) method for the
determination of multi-class antibiotics in chicken liver. The drugs under consideration were sulphonamide trimethoprim, tetracycline, chlortetracycline and tylosin. Sulfonamides exhibited higher response factors towards UV/VIS than mass spectrometer detection and the opposite was true for the rest of the analytes.

Rao et al (2015) developed a simple, sensitive, selective and precise HPLC method is developed for the determination of Oxytetracycline in bulk and honey samples. Separation of oxytetracycline was achieved on a KromosilC18 analytical column. The developed method was successfully applied for the determination of the oxytetracycline in honey sample following extraction of the oxytetracycline with McIlvaine buffer and Solid Phase Extraction system. All the honey samples that were analyzed for oxytetracyline residues had higher residue levels than the recommended maximum residue level for honey.

Zhou et al (2014) presented a method for the floral origin traceability of chaste honey and rape honey using high performance liquid chromatography–diode array detection–tandem mass spectrometry (HPLC–DAD–MS/MS) method. Kaempferol, morin and ferulic acid were used as floral markers to distinguish chaste honey from rape honey. Chromatographic fingerprinting at 270 nm and 360 nm could be used to characterize chaste honey and rape honey according to the analytical profiles. The results showed that chaste honey and rape honey could be successfully classified by their floral sources with the analytical methods developed through this study and could be considered encouraging and promising for the honey traceability from unifloral or multifloral nectariferous sources.

Wang et al (2014) developed a high-performing analytical technique for tetracycline detection. In this study, they developed a biosensor based on an indirect competitive enzyme-linked aptamer assay (ic-ELAA). A 76mer single-stranded DNA (ssDNA) aptamer, selected by Systematic Evolution of Ligands by Exponential Enrichment (SELEX), was applied for the recognition and detection of tetracycline in honey. The limit of detection was 9.6×10⁻³ ng/mL with a linear working range from 0.01 to 100 ng/mL toward TC in honey, and a mean recovery rate of 93.23% in TC-spiked honey was obtained.

Mahmoudi et al (2014) designed a study to investigate the occurrence of oxytetracycline residue in honey samples. A number of 145 honey samples were collected from Ardabil provinces (Northwest region of Iran). ELISA and HPLC were used to qualify and quantify the
contamination of the honey samples with oxytetracycline. The ELISA assay showed that out of 145 samples, 34 samples were positive for oxytetracycline residue. HPLC analyses confirmed the ELISA findings, although the level of oxytetracycline which detected in honey samples using HPLC method was remarkably (P<0.05) lower than that of which detected by ELISA.

**Fedorova et al (2014)**\(^{24}\) presented an analytical multiclass, multi-residue method for the determination of antibiotics in aquaculture products and validated. This method covers 32 antibiotics of different classes, which are frequently used in aquaculture. Three different extraction procedures were compared, and the extraction with acetonitrile (0.1 vol. % formic acid) showed the best results. The selected extraction procedure was validated at four different fortification levels (10 μg kg\(^{-1}\), 25 μg kg\(^{-1}\), 50 μg kg\(^{-1}\), and 100 μg kg\(^{-1}\)).

**Zai et al (2013)**\(^{25}\) stated that, the comparative evaluation of honey for the detection and quantification of antibiotics residues including tetracycline, streptomycin, gentamycin and penicillin using HPLC. A total of 100 samples were collected from market and categorized as branded and unbranded for comparative study. About 12.5% of branded sample and 19.96% unbranded samples were found positive. Tetracycline residue was found maximum in unbranded sample, Finally it was concluded that the unbranded honey had more contamination of antibiotic residues as compared with branded ones.

**Gumustas et al (2013)**\(^{12}\) reviewed about the application of Liquid chromatography (LC) methods for drug analysis. This review describes, the principles of ultra-high performance liquid chromatography and high performance liquid chromatography, validation of these methods, system suitability tests for the methods and application of methods for pharmaceutical analysis in the last 3 years.

**Alechaga et al (2012)**\(^{26}\) described a method based on ultra-high performance liquid chromatography (UHPLC) for the determination of chloramphenicol (CAP), its related compounds, thiamphenicol (TAP) and florfenicol (FF), in animal-derived foods (honey). For the coupling with mass spectrometry, heated-electrospray (H-ESI) is used as ionisation source improving vaporization efficiency. Two different sample treatments based on solid-phase extraction with mixed-mode cartridges for fish and meat products and hydrophilic–lipophilic-balanced cartridges for honey are proposed, providing limits of quantitation (LOQs) below μg kg\(^{-1}\) level.
Al-Waili et al (2012)\textsuperscript{11} reported that, the agricultural contamination with antibiotics is a challenging problem that needs to be fully addressed. Bee products, such as honey, are widely consumed as food and medicine and their contamination may carry serious health hazards. Presence of antibiotics residues might increase resistant human or animal’s pathogens. Many cases of infant botulisms have been attributed to contaminated honey. This article reviews the extent and health impact of honey contamination and stresses on the introduction of a strict monitoring system and validation of acceptable minimal concentrations of pollutants or identifying maximum residue limits for bee products, in particular, honey.

Perez et al (2012)\textsuperscript{27} created a database for the simultaneous analysis of more than 350 pesticides and veterinary drugs (including antibiotics) using ultra-high performance liquid chromatography coupled to high resolution Orbitrap mass spectrometry (UHPLC–Orbitrap-MS). This is a comprehensive exact mass database built using the Exactactive-Orbitrap analyzer. The developed database includes exact masses of the target ions and retention time data, and allows the automatic search of the included compounds. The presented database is suitable for qualitative analysis, and it was also evaluated for quantitative purposes in routine analysis, after the optimization and validation of a generic extraction method in honey samples.

Bie et al (2012)\textsuperscript{28} described a simple and effective method for the simultaneous determination of chlortetracycline (CTC), oxytetracycline (OTC), tetracycline (TC) and doxycycline (DC) in beehives using liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS). Analytes were extracted in EDTA-McIlvaine buffer from beehives by ultrasonication and shaking, purified using hexane distribution and HLB cartridges, quantified using a matrix-matched standard calibration curve, and validated according to Commission Decision 2002/657/EC and SANCO/10684/2009. The results showed that the developed method is sensitive and accurate and can be used to determine the levels of tetracycline antibiotics in beehives.

Ganguly et al (2011)\textsuperscript{8} presented data regarding antibiotic resistance and its global concern, which is particularly pressing in developing nations, including India, where the burden of infectious disease is high and healthcare spending is low. This report summarizes the situation as it is known regarding antibiotic use and growing resistance in India and recommends short and long term actions. Recommendations aim at reducing the need for antibiotics and eliminating
antibiotic use for growth promotion in agriculture and restricting the use of antibiotics for nontherapeutic uses in agriculture. These interventions should help to reduce the spread of antibiotic resistance, improve public health directly, benefit the populace and reduce pressure on the healthcare system.

Cronly et al(2010) developed a confirmatory method to allow for the analysis of chloramphenicol honey samples. Honey samples were first dissolved in water before a similar extraction. Honey extracts underwent a hexane wash to remove impurities. Honey extracts were evaporated to dryness and reconstituted in initial mobile phase. then injected onto a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system and analyzed in less than 9 min. Validation criteria of accuracy, precision, repeatability and reproducibility in honey.

Tylova et al (2010) described about the antibiotic residues present in soil due to animal manure. To reduce the usage of antibiotics in livestock industry the EU banned their application as growth promoters in 2006. Even though the antibiotics are still used for this purpose and therefore it is necessary to control their applications. An Ultra Performance Liquid Chromatography method (UPLC) with UV detection for determination of tetracycline (TC), in the liquid hog manure was developed. The antibiotics were extracted with ethyl acetate and separated on UPLC BEH Shield RP18 column. The validated method was selective for all analytes and system suitability was assessed.

Taokaenchan et al(2010) validated a high performance liquid chromatography (HPLC) method utilizing fluorescence detection was optimized and validated to determine tetracycline residues in honey. The separation of three tetracycline residues; oxytetracycline, tetracycline and chlortetracycline was carried out on a reverse–phase C8 column with a gradient elution. Fluorescence detection was observed at 518 nm (excitation wavelength at 393 nm) with 20 minute analysis time. The extraction with disodium ethylenediaminetetraacetic acid (Na2EDTA)-McIlvaine buffer pH 4 was performed and followed by HLB cartridge clean up step. The recoveries of oxytetracycline, tetracycline and chlortetracycline, at 50, 100 and 200 μg/kg spiked samples were higher than 80% for all compounds.

Vidal et al(2009) developed and validated for the simultaneous analysis of different veterinary drug residues (macrolides, tetracyclines, quinolones and sulphamides) in honey. The separation and determination was carried out by liquid chromatography couples to tandem mass
spectrometry (LC-MS?MS), using an electrospray ionization source (ESI) in positive mode. The method was validated and mean recoveries were evaluated at three concentration levels(10, 50 and 100µg/kg), ranging from 70-120% except for doxycycline, erythromycin and tylmicosin with recovery higher than 50 % at three levels.

Tamosiunas et al (2008)\textsuperscript{17} performed the determination of ten sulfonamides (SAs) in honey has been compared using column liquid chromatography (LC) and ultra-performance liquid chromatography (UPLC) coupled to tandem mass spectrometry (MS–MS). A liquid–liquid extraction with acetonitrile followed by solid-phase extraction on a Strata-X cartridge was developed for sample preparation. Using UPLC the separation time was shortened about 30% reducing the run time by 8 min and a better resolution was achieved compared to LC. For a majority of the spiked compounds, UPLC gave significantly better precision.

Pan C et al., (2006)\textsuperscript{32} developed a HPLC method with a monolithic column and detection by mass spectrometry for the determination of chloramphenicol in honey samples previously cleaned by use of a modification of the QuEChERS procedure. Chloramphenicol (CAP) residues at ppb concentrations were detected by liquid chromatography–mass spectrometry (LC–MS), with electrospray ionization, in negative-ion mode. So, the method was fit for the purpose of monitoring commercial products or checking MRL compliance.

Gallina et al (2005)\textsuperscript{33} presented an uncontrolled use of antibiotics such as sulphonamides & streptomycin. The optimization of analytical methods for the detection of residues of antibacterial drugs like streptomycin in honey was pursued and the methods applied to routine samples. Streptomycin was investigated by liquid chromatographic techniques with a post column reaction and fluorimetric detection. The validation parameters obtained with these methods were 88% for recovery 10ng/g as quantification limits for streptomycin.

Pena et al (2005)\textsuperscript{6} developed an analytical method for the determination of Oxytetracycline and Tetracycline residues in honey. The method was validated according to the guidelines laid down by the 2002/657/EC European Decision parameters: decision limit and detection capability were 20 and 21 µg/kg and 49 and 50 µg/kg for Oxytetracycline and Tetracycline, respectively, and recoveries of OTC and TC from spiked samples, at three fortification levels, were higher than 87% for both compounds. The analytical method was applied to 57 honey samples.
Bogiali et al (2005)\textsuperscript{34} described a simple, selective and sensitive procedure for determining nine widely used aminoglycoside antibiotics (AGs) in bovine whole milk. It is based on matrix solid-phase dispersion followed by liquid chromatography (LC)–tandem mass spectrometry (MS) using an electrospray ion source. After acidification and filtration, 0.2 ml of the aqueous extract was injected into the LC column. MS data acquisition was performed in the multi reaction monitoring mode. They are well below the tolerance levels set by both the European Union and the U.S. Food and Drug Administration.

Ortelli et al (2004)\textsuperscript{35} reported about the use of antibiotics in bee keeping to control European and American Foulbrood. The broad spectrum antibiotics chloramphenicol have been used for the curative purposes in veterinary medicine, but is now forbidden in numerous countris, although still used in South–East Asia. A LC-MS/MS has been developed for the analysis of sub µg/kg residues of chloramphenicol in honey. The result was found to be positive for the collected sample, which shows that method was accurate for the analysis of chloramphenicol.

Pagliuca et al (2002)\textsuperscript{36} described how to minimize American foulbrood disease (\textit{Paenibacillus larvae larvae}) in honeybees (\textit{Apis mellifera} L.), by using oxytetracycline (OTC) in beekeeping in many countries. This means a potential risk of toxic or allergic reactions for hypersensitive people (World Health Organisation, 1969). For this reason, a simple method for the detection of OTC residues in honey by high performance liquid chromatography (HPLC) coupled with ultraviolet-visible spectrophotometry using a photodiode array detector (HPLC-DAD).