CHAPTER II

REVIEW OF LITERATURE
2.0. REVIEW OF LITERATURE

2.1. AMPICILLIN

Antibiotic, ampicillin is given to livestock by veterinarians for the prevention and treatment of disease and also as a stimulant for animal growth and conversion. In spite of the way the antibiotics are inserted into the body of the animal, there will always be residual risk in milk which exceeds the maximum level allowed. Thus, it can lead to health disorders in humans and in some cases even death.

The literature survey has revealed several techniques for the determination of ampicillin residues in milk. They include high-performance liquid chromatography-electrospray mass spectrometry (Straub and Voyksner, 1993; Niu and Zhu, 2001; Susan et al., 2003; Ghidini et al., 2003; Sara et al., 2004; Helio et al., 2007), high-performance liquid chromatography with integrated pulsed amperometric detection (Dasenbrock and Lacourse, 1998), high-performance liquid chromatography with fluorescence detection (Luo et al., 1997), ion-pair reversed phase high performance liquid chromatography (Verdon and Couedor, 1996), high-performance liquid chromatography with diode array detection, microbial receptor assay method (Anderson et al., 1996), planar chromatography (Abjean and Lahogue, 1997), receptor-based lateral-flow rapid one-step assay (Robert et al., 2011), improved tube test method (Anakalo and Gathoni, 2004), electrophoretic method coupled with bioautography (Cutting et al., 1995), micellar electrokinetic capillary chromatography (Rade et al., 2009), microbial assay method (El-Shorbagy et al., 2009). The above said methods suffer from disadvantages like use of expensive detectors, use of internal standard and cumbersome procedures. Hence they are not applied for routine analysis of ampicillin residues in milk. The method reported by Luo et al., (1997) is applied only to animal tissues.
Only a few high performance liquid chromatographic methods with ultra detector (Marie et al., 1998; Roseane et al., 2006; Khaskheli et al., 2008; Torlak et al., 2012; Zana et al., 2012) have been reported for the determination of ampicillin residues in milk. The reported high performance liquid chromatographic methods with ultra detector suffers from disadvantages such as lack of sensitivity, use of internal standard, longer retention time and derivitization of the residue before analysis.

Taking these points into consideration, in the present study an attempt has been made to develop a simple, sensitive, precise and accurate high performance liquid chromatographic method using ultra violet detector (method M1) for the determination of ampicillin residues in milk and milk products.

2.2. DICLOFENAC SODIUM

Diclofenac, an antibiotic which is used in bovine species for anti-inflammatory, analgesic and antipyretic therapy via intramuscular injection. They are also used as adjuncts to antibacterial treatment of respiratory disease of calves. After the drug has been inserted into the animal body, there will always be drug residual in milk. Sometimes the drug residues exceed the maximum level allowed which may lead to health disorders in humans.

Davarani et al. (2012) extracted sodium diclofenac as an acidic compound from the bovine milk with electro membrane extraction method. The extract was then analyzed by HPLC with UV-detection for quantification of sodium diclofenac. Amber et al. (2009) determined diclofenac sodium in milk samples using capillary electrophoretic method. Gas chromatography-tandem mass spectrometry (Dowling et al., 2008) and liquid chromatography tandem mass spectrometry methods (Dowling et al., 2009; Dubreil et al., 2011) have been developed to analyze diclofenac residues in bovine milk.

A highly sensitive and specific indirect competitive enzyme-linked immunosorbent assay (ELISA) for the determination of diclofenac in tap and
surface water samples as well as wastewater collected at 20 sewage treatment plants in Austria and Germany was developed by Anping et al. (2003). HPLC (Nief and Suhaib, 2011) and gas chromatography-hybrid chemical ionization mass spectrometry (Agueraa et al., 2006) methods have been applied for the determination of diclofenac sodium in environmental water samples.

The thorough literature survey has revealed that no HPLC with UV detection method has been reported for the determination of diclofenac sodium residues in milk. Therefore, in the present investigation an attempt is made to develop a simple, sensitive and cost effective HPLC method (method M2) for the determination of diclofenac sodium residues in milk.

2.3. CHLORTETRACYCLINE

Chlortetracycline is commonly used all over the world with broad antibacterial spectrum and bacteriostatic activity, as medicines for animals and as feed additives because they are well absorbed, exhibit low toxicity and are relatively inexpensive. The chlortetracycline residues in milk, meat, egg may have adverse effects on public health when they are present above its maximum residue levels.

HPLC with UV (Georg et al., 2000), fluorescence (Georg et al., 2000) & MS-MS detection (Georg et al., 2000; Alfredsson et al., 2005) and microbiological (Meredith et al., 1965; Katz and Fassbender, 1970) methods were reported for the quantification of chlortetracycline residues in eggs, honey and poultry tissues. TLC densitometry with fluorescence detection and HPLC with mass spectrometry were reported by Naidong et al., (2003) and Guo et al., (2012), respectively for the determination of chlortetracycline in animal feeds.

The literature review reveals that several analytical methods have been established for the determination of chlortetracycline residues in milk. They include HPLC with fluorescence detection (Siska and Carlos, 1994; Mehran et al., 2011), HPLC with diode-array detection (Cinquina et al., 2003), HPLC with mass
spectrometry (Nakazawa et al., 1999; De Ruyck and De Ridder, 2007), HPLC with
coulometric electrode array system (Funian et al., 2004), UPLC with
photodiode array detection (Lian and Li, 2009), internal reflected resonance light
scattering (Ping et al., 2001), microbiological methods (Deborah et al., 1998;
Kurittu et al., 2000; Montero et al., 2005), capillary electrophoresis with mass
spectrometry (Wang et al., 2007), capillary electrophoresis with UV detection
(Chen and Gu, 1995; Ibarra et al., 2011), high-voltage electrophoresis (Krcmár
and Růžicková, 1996) and voltammetry (Agúi et al., 2003). Most of the reported
methods require costly detectors, cumbersome procedures and long time for the
single analysis. Hence they are not applicable for the routine analysis of
chlortetracycline residues in milk samples.

Several methods for the determination of chlortetracycline residues in
meat samples have been described via HPLC with UV detection (Korimová et al.,
1997; Kanda et al., 2008 Mehran et al., 2009), HPLC method with fluorescence
detection (Katia et al., 1998; Angelina et al., 2007; Marilyn et al., 2007), HPLC
with tandem mass spectrometry (Katia et al., 1998; Angelina et al., 2007; Kanda
et al., 2008; Thais and Francisco, 2011; Cetinkayaa et al., 2012), HPLC with
photodiode array detection (Biswas et al., 2007), fluorimetry (Honikel and
Hambloch, 1976), enzyme linked immunosorbent assay (Katia et al., 1998),
electrochemical immunosensor (Faridah et al., 2012) and microbiological
(Korimová et al., 1997; Katia et al., 1998; Lieve et al., 2001) methods.
Julie et al. (2003) and Ran et al. (2010) reported HPLC with detection for the
determination of chlortetracycline residues in pig faeces and pig solid manure,
respectively. A kinetic spectrophotometric method has been developed by
Yongnian et al. (2010) for estimation of chlortetracycline in feed and muscle
samples.

To the best of our knowledge there is only one HPLC with ultraviolet
detector method (Pavlína et al., 2009) is proposed for the quantification of
chlortetracycline residues in milk. This method (Pavlína et al., 2009) lacks the sensitivity, selectivity, precision and accuracy.

Only two methods have been reported in the literature for the determination of chlortetracycline residues in pork. They include HPLC method with fluorescence detection (Katia et al., 1998) and HPLC with tandem mass spectrometry (Katia et al., 1998). Though these methods are sensitive, they require sophisticated detectors.

In the present investigation the author has made an attempt to develop two simple, sensitive and cost effective HPLC methods (methods M3 and M7) for the determination of chlortetracycline residues in milk (method M3) and pork (method M7).

2.4. SULFADIMIDINE

Sulfadimidine is inexpensiveness with wide-spectrum antimicrobial activity. Hence sulfadimidine is most commonly used in veterinary practices. However, the presence of residues of sulfadimidine in milk is of toxicological and regulatory concern as it could be carcinogenic and cause allergic hypersensitivity reactions in human beings. Therefore, an analytical method for monitoring sulfadimidine residues is required.

Nouws et al. (1989) studied the pharmacokinetics and residues of sulfadimidine and its metabolites in horses, calves, cows, and laying hens by employing specific HPLC methods. Microbiological (Hussein et al., 2005), HPLC (Roudaut and Garnier, 2002; Koarova et al., 2004) and spectrophotometric (Romváry and Simon, 1992) methods are reported in the literature for the determination of sulfadimidine residues in eggs. HPLC with pre-column derivatization (Zou et al., 2007), liquid chromatography-electrospray-mass spectrometry (Fuh and Chan, 2001) and capillary electrophoresis-electrospray-mass spectrometry (Soto et al., 2007) methods are reported for the quantification
of sulfadimidine residues in meat samples. None of the above reported methods were applied for the determination of sulfadimidine residues in milk.

Few analytical methods have been developed for the determination of sulfadimidine residues in milk (Weber, 1990; Van Poucke et al., 1991; Smedley and Takeda et al., 1992; Van Rhijn et al., 2002). They include HPTLC (Van Poucke et al., 1991), liquid chromatography-electrospray-mass spectrometry (Van Rhijn et al., 2002), HPLC with fluorescence detector (Takeda et al., 1992) and HPLC with UV detector (Smedley and Weber, 1990). Earlier methods for measuring the sulfadimidine residues in milk were found to be unsuitable due to lack of sensitivity, selectivity, requires expensive detector, cost involved in the required instrumentation is high, use of internal standard and requires derivitization of the residue. Hence in the present study an attempt has been made to develop and validate a simple, sensitive and cost effective HPLC with UV detection (method M4) for the determination of residues of sulfadimidine residues in milk.

2.5. DOXYCYCLINE

Tetracycline antibiotics have served as important class of veterinary drugs for many years. One representative of that class is doxycycline. When the withdrawal periods are not obeyed, the antibiotic residues may be present in edible products, e.g., in meat, eggs or milk. The residues of doxycycline in edible products may results in harmful effects on humans. Hence there is a need of an analytical method for the determination of doxycycline residues.

A number of methods like HPLC with diode-array detection (Cinquina et al., 2003; Denobile et al., 2004; Samanidou et al., 2007), HPLC with fluorescence detection (Mehran et al., 2011), HPTLC (Choma et al., 1999), microbial (Nouws et al., 1998; Kurittu et al., 2000) and capillary electrophoresis (Chen and Gu, 1995; Ibarra et al., 2011) have been reported in the literature for the determination of doxycycline in bovine milk. HPLC with fluorescence detection (Narin and
Supaporn, 2008) and capillary zone electrophoresis (Casado et al., 2007) methods were proposed for determination of doxycycline residues in honey. However, the above said methods were not applied to the determination of doxycycline in chicken.

Literature survey reveals that HPLC methods with fluorescence detection (De Wasch et al., 1998), UV detection (Gajda et al., 2013) and tandem mass spectrometry (Gajda et al., 2013) have been reported for the estimation of doxycycline residues in chicken. Although above methods reported are found to be sensitive, they use costly detection techniques, fluorescence detection & tandem mass spectrometry, and involve tedious & time consuming sample preparation steps. HPLC with UV detection requires an internal standard. Hence it was envisaged to develop a simple, sensitive, cost effective and reliable HPLC method with UV detection (method M5) for estimation of doxycycline residues in chicken without the use of internal standard.

2.6. SULFADIMETHOXINE

Sulfadimethoxine is extensively used as veterinary medicine for preventive and curative purpose. The residues of sulfadimethoxine are occasionally observed in carcasses at slaughter. This may be due to dosage and/or duration of treatment not being in compliance with the doctor’s recommendations, which probably induces residue levels in the meat above the maximum residue limit.

In order to determine the sulfadimethoxine residues in milk, several methods have been developed, including HPLC with UV detection (Thomas et al., 2004; Sun and Zhao, 2007), HPLC method with diode-array detection (Long et al., 1990; Furusawa, 2000; Kunihiro and Furusawa, 2004; Yin et al., 2007a; Yin et al., 2007b; Evanthia et al., 2011; Tolika et al., 2011), HPLC with fluorescence detection (Laloux et al., 1996; Marques, 2008), HPTLC (Van Poucke et al., 1991), liquid chromatography with tandem mass spectrometry (Chiara
et al., 2003; Sara et al., 2003; Clark et al., 2005) and microbial assay (Montero et al., 2005; Linage et al., 2007; Hyun et al., 2009; Sierra et al., 2009).

The literature is enriched with several methods for determination of sulfadimethoxine residues in egg including HPLC with UV detection (Roudaut and Garnier, 2002), HPLC with photo diode array detection (Furusawa, 2002; Kunihiro and Furusawa, 2005) gas chromatography with mass selective detection (Tarbin et al., 1999), liquid chromatography with mass spectrometry (Tarbin et al., 1999; Chiara et al., 2003; Forti and Scortichini, 2009). Few liquid chromatographic methods were reported for the analysis of sulfadimethoxine residues in muscle tissue of cat fish (George et al., 1987; Long et al., 1990; Walker and Barker, 1994; Du et al., 1995; Theresa et al., 2006), salmon (Kitts et al., 1995; Theresa et al., 2006), chickens (George et al., 1987) and shrimp (Theresa et al., 2006). Biochip array technology (Ionela et al., 2012) and microbial assay (Gaudin et al., 2013) methods were reported for the determination of sulfadimethoxine in honey.

Several analytical methods are currently available for the determination of sulfadimethoxine residues in meat. They include HPLC with fluorescence detection (Takeda and Akiyama, 1991), HPTLC (Van Poucke et al., 1991), liquid chromatography with tandem mass spectrometry (Boison and Keng, 1995), capillary zone electrophoresis (Soto et al., 2006; Soto et al., 2007; Ting et al., 2008; Muhammad et al., 2009; Rodrigo et al., 2009).

To the best of our knowledge there are no HPLC reports on the determination of sulfadimethoxine residues in goat meat (mutton). Hence, in the present study an attempt has been made to develop a simple, sensitive, cost effective and reliable HPLC with UV detection (method M6) for estimation of sulfadimethoxine residues in goat meat (mutton) without the use of internal standard.
2.7. NALIDIXIC ACID

Because of its high potency against bacteria, nalidixic acid is used to treat bacterial infections in fish farming. The drug is usually administered to fish mixed with feed. The administration of nalidixic acid to fish, intended for human consumption, has become a serious problem because their residues can persist in edible fish tissues. Therefore, an analytical method is required for the determination of the nalidixic acid residues.

Ute and Anke (2007), Christine et al. (2007) and Dufresne et al. (2007) reported HPLC with fluorescence detection and HPLC with mass spectrometry methods for the determination of nalidixic acid residues in shrimp. HPLC with fluorescence detection (Yorke and Froc, 2000; Eric et al., 2005; Stoilova and Petkova, 2010; Takeda et al., 2011) devoted to assay of nalidixic acid in chicken muscle are reported. Analytical techniques applicable to nalidixic acid determination in human serum, plasma and urine samples, which include HPLC with UV detection (Boppana and Swanson, 1982), HPTLC (Hundt and Barlow, 1981), spectrofluorimetry (Moghbel and Makhmalzadeh, 2006), capillary zone electrophoresis (Pérez et al., 1999) and voltametry (Ibrahim et al., 2002) are reported. Chromatographic methods for the determination of nalidixic acid residues in milk (Christodoulou and Samanidou, 2007), honey (Durden and Fernandes, 2010) and swine kidney (Toussaint et al., 2002) were also reported.

Nalidixic acid residues in fish are estimated using HPLC with fluorescence detection (Horie et al., 1987; Ikai et al., 1989; Munns et al., 1998; Eric et al., 2005; Takeda et al., 2011), liquid chromatography-tandem mass spectrometry (Dufresne et al., 2007; Li et al., 2009) and fluorometry (Mei et al., 2002). Though the reported methods are sensitive, they require expensive detectors and cumbersome procedure. Determination of nalidixic acid residues in fish using HPLC with UV detection is not reported till now. Therefore, an attempt has been made to develop a simple, sensitive, cost effective and reliable HPLC with UV
detection (method M8) for estimation of nalidixic acid residues in fishes without the use of internal standard.

8. CIPROFLOXACIN

Ciprofloxacin is a widely used antibiotic in livestock and fish farm industries. The analysis of the residual amounts of ciprofloxacin is of importance for the control of the quality of the food products for the consumer. Hence there is a need of an analytical method for the estimation of ciprofloxacin residues in the food products.

Ciprofloxacin was determined in milk by HPLC with diode-array detection (Cinquina et al., 2003; Hermo et al., 2008), HPLC with fluorescence detection (Guixiang et al., 2005; Chonan et al., 2008; Hermo et al., 2008), liquid chromatography–tandem mass spectrometry (Hoofa et al., 2005; Hermo et al., 2008; Chui et al., 2010), capillary electrophoresis-tandem mass spectrometry (Lara et al., 2006), capillary electrophoresis with UV detection (Piñero et al., 2012), capillary electrophoresis with laser induced fluorescence detection (Manuel et al., 2010) and Enzyme-linked immunosorbent assay (Bin et al., 2010). Ciprofloxacin was determined in chicken muscle tissues by immunoaffinity chromatography (Sijun et al., 2009), HPLC with fluorescence detection (Marilyn, 2001; Zhao et al., 2007; Chonan et al., 2008), HPLC with diode-array detection (Moema et al., 2012), liquid chromatography–tandem mass spectrometry (Clemente et al., 2006; San et al., 2007; Chui et al., 2010), HPLC with UV detection (Clemente et al., 2006).

There are reports on the determination of ciprofloxacin residues in prawn (Chonan et al., 2008), rainbow trout (Chonan et al., 2008), eggs (Durden and MacPherson, 2007; Chonan et al., 2008; Blasco and Picó, 2012), animal feed (Borràs et al., 2012; Małgorzata et al., 2013), pork (Chui et al., 2010), shrimp (Chui et al., 2010), cattle (Ralph et al., 2009), pig (Toussaint et al., 2005; Ralph et al., 2009), turkey (Ralph et al., 2009), rabbit (Ralph et al., 2009) and water...
These methods include HPLC with fluorescence detection (Chonan et al., 2008; Pena et al., 2010; Gil et al., 2012; Mamdouh et al., 2013), liquid chromatography-tandem mass spectrometry (Toussaint et al., 2005; Durden and MacPherson, 2007; Chui et al., 2010; Blasco and Picó, 2012), turbulent flow chromatography-tandem mass spectrometry (Ralph et al., 2009), electrochemical assay (Mamdouh et al., 2013) and capillary electrophoresis (Herrera et al., 2010).

Few methods are reported for the determination of ciprofloxacin residues in fish. They include HPLC with fluorescence detection (Mi-Ra et al., 2006; Zhang et al., 2010), HPLC with photodiode array detection (Evaggelopoulou and Samanidou, 2013), Liquid Chromatography-tandem mass spectrometry (Chui et al., 2010) and electrochemical method (Mamdouh et al., 2013). The reported methods suffer from disadvantages like use of internal standard, tedious procedures and require highly sophisticated instrumentation. Hence, in the present study an attempt has been made to develop a simple, sensitive, cost effective and reliable HPLC with UV detection (method M9) for estimation of ciprofloxacin residues in fish with out the use of internal standard.

2.9. CHLORAMPHENICOL

Chloramphenicol is commonly used in shrimp culture and to treat honey bees as it exhibits broad spectrum activity over various microorganisms. Application of chloramphenicol in shrimp culture and honey bees is raising serious concerns due to its accumulation in shrimp tissues and honey. A rapid analytical method for the assay of chloramphenicol residues in shrimp tissues and honey is needed to ensure the safety and adequacy of the food supply.

There are reports on the liquid chromatographic methods for the determination of chloramphenicol residues in swine [muscle and liver] (Li et al., 2002; Cerkvenik, 2006), milk (Acacia et al., 2003; Philippe et al., 2004;
Pan et al., 2005; Rodziewicz and Zawadzka, 2008; Mónica et al., 2009) and chicken [liver, kidney and muscle] (Tajik et al., 2010). Li et al., (2010), Zhenbo and Jun (2011) and Suchada et al. (2008) described enzyme linked immunosorbent assay, voltammetric electronic tongue system and cyclic voltammetric methods, respectively for the estimation of chloramphenicol in milk.

The chloramphenicol residues in shrimps can be detected by several methods such as liquid chromatography-mass spectrometry (Barbara et al., 2002; Joe et al., 2003; Ramos et al., 2003; Sandra et al., 2003; Evangelos et al., 2004; Tyagi et al., 2008; Rocha et al., 2009; Caroline et al., 2013), enzyme linked immunosorbant assay (Sandra et al., 2003), gas chromatography–tandem mass spectrometry (Sandra et al., 2003), gas chromatography with electron capture detection (Munns et al., 1994), surface plasmon resonance biosensor assay (Dumont et al., 2006), microbial assay (Shakila et al., 2007), immunoassay (Meng et al., 2012). The aforesaid methods require expensive detector, laborious procedure and long time for analysis. So they are not applied for the routine analysis of chloramphenicol residues. Only two HPLC with UV detection (Yang et al., 2004; Xizhi et al., 2007) are reported for the determination of chloramphenicol residues in shrimps. The reported HPLC with UV detection methods are insufficiently sensitive, less accurate and precise.

The chloramphenicol residues has been determined in honey by a variety of analytical techniques such as enzyme linked immunosorbent assay (Hao and Hai, 2005), gas chromatography with electronic capture detection (Hao and Hai, 2005), gas chromatography-mass spectrometry (Hao and Hai, 2005), liquid chromatography-tandem mass spectrometry (Verzegnassi et al., 2003; Jing et al., 2006; Rønning et al., 2006; Tsuyoshi et al., 2012; Caroline et al., 2013), immunoassay (Meng et al., 2012) and chemiluminescence immunoassay (Yi et al., 2012). To the best of our knowledge, there is only one report for the determination of chloramphenicol residues in honey by HPLC with UV detection.
(Hao and Hai, 2005) method. The reported HPLC with UV detection method lacks the required sensitivity, accuracy and precision.

Taking these points into consideration, in the present investigation an attempt has been made to develop two simple, sensitive, precise and accurate HPLC methods using ultra violet detector for the determination of chloramphenicol residues in shrimp tissues (method 10) and honey (method 17).

2.10. ENROFLOXACIN

Enrofloxacin is a synthetic antibiotic that is widely used in veterinary medicine. Enrofloxacin residues may remain in tissues, milk, egg, etc. intended for human consumption. Therefore, analytical methods are needed to determine them in biological samples.

The literature is enriched with several methods for the determination of enrofloxacin residues in Nile tilapia (Weihai et al., 2006), swine [liver (Chen and Li, 2013) & muscle (Hatano et al., 2004; Sijun et al., 2007; Takeda et al., 2011)], chicken [blood (Victoria et al., 2005), serum (Marilyn et al., 2007), liver (Takeda et al., 2011), & muscle (Chen and Schneider, 2003; Hatano et al., 2004; Schneider, 2004; Anne et al., 2006; Sijun et al., 2007; Junxia et al., 2009; Zhao et al., 2007; Članjak et al., 2011; Jin et al., 2011; Takeda et al., 2011)], pig [kidney (Anne et al., 2006; Manuel et al., 2010), muscle (Li et al., 2009) & plasma (Hernandez, 2002)], eggs (Lolo et al., 2005; Anne et al., 2006; Takeda et al., 2011; Anna et al., 2012), fish (Anne et al., 2006; Li et al., 2009), bovine [muscle (Hoof et al., 2005), kidney (Myllyniemi et al., 2002) & plasma (Tyczkowska et al., 1994)], milk (Hormazabal and Yndestad, 1994; Tyczkowska et al., 1994; Cinquina et al., 2003; Hatano et al., 2004; Marazuela and Moreno, 2004; Hoof et al., 2005; Francisco et al., 2006; Rodríguez et al., 2006; Hermo et al., 2008; Zhou et al., 2008; Blasco et al., 2009; Tong et al., 2010; Manuel et al., 2010; Pavlína et al., 2011; Turnipseed et al., 2011; Tian, 2011; Piñero et al., 2012), prawn (Hatano et al., 2004), broiled eel (Hatano et al., 2004), salmon
processed foods [ham (Takeda et al., 2011), sausage (Takeda et al., 2011) & fish sausage (Takeda et al., 2011)], fish farming water (Hanwen et al., 2011), fish feed (Hanwen et al., 2011) and aquacultured products (Hoof et al., 2005).

The methods include HPLC with mass spectrometry (Turnipseed et al., 2003; Hatano et al., 2004; Hoof et al., 2005; Lolo et al., 2005; Weihai et al., 2006; Marilyn et al., 2007; Hermo et al., 2008; Li et al., 2009; Turnipseed et al., 2011; Tian, 2011; Anna et al., 2012), HPLC with fluorescence detection (Hormazabal and Yndestad, 1994; Marazuela and Moreno, 2004; Marilyn et al., 2007; Sijun et al., 2007; Zhao et al., 2007; Hermo et al., 2008; Pavlína et al., 2011; Takeda et al., 2011; Anna et al., 2012), HPLC with UV detection (Marazuela and Moreno, 2004; Victoria et al., 2005; Hermo et al., 2008), HPLC with diode array detection (Cinquina et al., 2003; Hanwen et al., 2011), HPLC with luminescence detection (Rodríguez et al., 2006), ion-pairing liquid chromatography (Tyczkowska et al., 1994), indirect competitive fluorescence-linked immunosorbent assay (Junxia et al., 2009), enzyme-linked immunosorbent assay (Anne et al., 2006; Jin et al., 2011; Članjak et al., 2011), microbial assay (Članjak et al., 2011), capillary electrophoresis with electrochemiluminescence detection (Zhou et al., 2008), capillary electrophoresis with UV detection (Hernandez, 2002; Piñero et al., 2012), capillary zone electrophoresis with mass spectrometry (Francisco et al., 2006; Blasco et al., 2009), capillary electrophoresis with laser induced fluorescence detection (Manuel et al., 2010), conductimetry (Myllyniemi et al., 2002), fluorescence assay (Chen and Schneider, 2003; Schneider, 2004, Tong et al., 2010) and luminescence method (Chen and Li, 2013).

Methods for the determination of enrofloxacin residues in shrimps which have been reported previously included HPLC with fluorescence detection (Sijun et al., 2007; Takeda et al., 2011), HPLC with mass spectrometry (Li and Kijak, 2011), enzyme-linked immunosorbent assay (Anne et al., 2006; Liu et al., 2009).
and microbial assay (Dang et al., 2010). However the reported methods suffer from disadvantages such as use of costly detectors, cumbersome procedure, less accurate and require long time for the analysis. To the best of our knowledge there are no reports on the determination of enrofloxacin residues in shrimp using HPLC with UV detection method.

Hence, it was made an attempt to develop a simple, sensitive, cost effective and reliable HPLC with UV detection (method M11) for estimation of enrofloxacin residues in shrimp without the use of internal standard.

1.11. FENVALERATE

Fenvalerate is extensively used as pest control agent in agricultural production because of their selective insecticidal activity. Fenvalerate residues in vegetables and fruits after application to the crops still pose risks to human health. Therefore, monitoring of fenvalerate residue levels in vegetables and fruits is of particular concern for human health.

The techniques reported for the assay of fenvalerate residues in fruits and vegetables include HPLC with fluorescence detection (López et al., 2001; Vázquez et al., 2008), HPLC with UV detection (Pang et al., 1995; Suthasinee et al., 2012), HPLC with chemiluminescence detection (Galera et al., 2006), liquid chromatography-tandem mass spectrometry (Pang et al., 1987), gas chromatography-mass spectrometry (Pang et al., 1987; Jain, 1996; Ramesh and Balasubramanian, 1998; Sannino et al., 2003; Tahir et al., 2011), gas chromatography with electron capture detection (Pang et al., 1994a; Pang et al., 1994b; Pang et al., 1995; Ramesh and Balasubramanian, 1998, Sannino et al., 2003; Seyed and Somashekar, 2010; Shinger et al., 2012) and immunoaffinity chromatography (Wang et al., 2011). Enzyme-linked immunosorbent assay was described by Juan et al., (2010) for the detection of fenvalerate in environmental water samples. Urmila et al., (2012) and Kumar et al., (2006) proposed simple spectrophotometric methods for the determination of fenvalerate in their
formulations and environmental samples. Photoelectrochemical sensor and capacitive chemical sensor for fenvalerate assay were developed by Yanhu et al. (2013) and Ji-Lai et al. (2004).

A few methods have been previously reported for the determination of fenvalerate residues in grapes. They include HPLC with UV detection (Suthasinee et al., 2012), gas chromatography-mass spectrometry (Pang et al., 1987; Tahir et al., 2011), liquid chromatography-tandem mass spectrometry (Pang et al., 1987) and gas chromatography with electron capture detection (Pang et al., 1995; Seyed and Somashekar, 2010). The aforesaid methods (Pang et al., 1987; Pang et al., 1995; Seyed and Somashekar, 2010; Tahir et al., 2011) have the disadvantages like requirement of expensive detector system and use of cumbersome procedures. The reported high performance liquid chromatographic methods with UV detection (Suthasinee et al., 2012) suffers from disadvantages such as lack of sensitivity, precision & accuracy, use of internal standard and longer retention time. To the best of our knowledge, there are no reports on the HPLC with UV detection method for the assay of fenvalerate residues in the mango.

Taking these points into consideration, an attempt has been made to develop a simple, sensitive, precise and accurate reverse phase high performance liquid chromatographic method using ultra violet detector (method M12), with out the use of internal standard, for the determination of fenvalerate residues in grapes and mango.

2.12. METHYL PARATHION

Due to the presence of significant amount of nutrients and minerals, fresh fruits are the important part of a healthy diet. In order to increase the world food production the use of pesticides in agriculture has increased. Among various pesticides, methyl parathion is widely used to control undesirable weeds, moulds, insects and pests. The use of methyl parathion has resulted in occurrence of
methyl parathion residues and their metabolites in air, water, soil, crops, vegetables and fruits. The fruits and vegetables contaminated with methyl parathion residues (beyond the maximum residue level) when consumed by human turned out to be toxic. Therefore, an analytical method is required to determine the concentration of methyl parathion residues in biological and environmental samples.

Several analytical techniques for the determination of methyl parathion residues in vegetables, fruits and environmental water samples have been appeared in literature including HPLC with UV detection (Flemming, 1981; Martinez et al., 1992; Luis et al., 1997; Wang et al., 1999; Guangming et al., 2002), gas chromatography with mass spectrometry (Fytianos et al., 2002; Liapis et al., 2003; Zhou et al., 2006; Shanker et al., 2010), amperometry (Gale et al., 1985), gas chromatography with thermionic specific detector (Cabrera et al., 2000), gas chromatography with nitrogen phosphorous detection (Fytianos et al., 2006), fluorescence polarization immunoassay (Anna et al., 2003), HPTLC-enzyme inhibition assay (Akkad and Schwack, 2010; Akkad and Schwack, 2011; Akkad and Schwack, 2013), cholinesterase-inhibition assay (Neufeld et al., 2010), HPLC with mass spectrometry (Fernández et al., 2001), low-temperature plasma ambient ionization mass spectrometry (Joshua et al., 2010), enzyme-linked immunosorbent assay (Min et al., 2003; Lipeng et al., 2013), voltametry (Bourque et al., 1989; Lucas et al., 1993; Mohammed et al., 2007; Xingyuan, 2011) and spectrophotometry (Toral et al., 2002; Nagaraja and Bhaskara, 2007; Neetu et al., 2013).

The methods (Flemming, 1981; Martinez et al., 1992; Anna et al., 2003; Fytianos et al., 2006; Joshua et al., 2010; Xingyuan, 2011; Akkad et al., 2012) reported for the determination of methyl parathion residues in fruits suffers from one or more disadvantages like more laborious, require highly specialized technicians and expensive instruments. For this reason, it was made an attempt to develop a simple, sensitive, cost effective and reliable HPLC with UV detection.
(method M13) for estimation of methyl parathion residues in fruits (mango and grapes) without the use of internal standard.

2.13. CHLORPYRIFOS

The vegetable growers have been using pesticides frequently to have the higher yield. But the overdose of pesticides makes the residue problem, which might pollute the food and be harmful for our health. Analytical methods are needed to determine, quantify and confirm pesticide residues in vegetables for both research and regulatory purposes.

Detailed survey of literature for chlorpyrifos revealed several methods have been reported for the assay of chlorpyrifos residues in different fruits, vegetables and water samples. These analytical techniques include HPLC with UV detection (Richard et al., 2006; Sajjad et al., 2009; Cozma et al., 2011; Devendra et al., 2011; Barkat et al., 2012; Paranthaman et al., 2012; Shailendra et al., 2012; Alamgir et al., 2013; Tordzagla et al., 2013), liquid chromatography-tandem mass spectrometry (Steven et al., 2005; Rohan et al., 2012), HPTLC (Iqbal et al., 2007; Yue et al., 2008; Akkad and Schwack, 2012; Rouhollah et al., 2012), gas chromatography-mass spectrometry (Steven et al., 2005; Paranthaman et al., 2012; Tomas et al., 2012), gas chromatography with electron capture detection (Mohammad et al., 2010; Subhash et al., 2010; Devendra et al., 2011; Shailendra et al., 2012), spectrophotometry (Venugopal et al., 2012), reflectance near-infrared spectroscopy (Umesh et al., 2012), chemiluminescence assay (Aifang et al., 2008), amperometric immunosensor assay (Sun et al., 2012a; Sun et al., 2012b), immunoassay (Gabaldón and Maquieira, 2007) and capillary electrochromatography (Weimin et al., 2010).

Only few methods have been reported for the determination of chlorpyrifos residues in cabbage, cauliflower and capsicum. The methods adopted include HPLC with UV detection (Barkat et al., 2012; Shailendra et al., 2012), gas chromatography with electron capture detection (Subhash et al., 2010;
Shailendra et al., 2012), amperometric immunosensor assay (Sun et al., 2012a) and capillary electrochromatography (Weimin et al., 2010). The methods reported for analysis of chlorpyrifos residues in cabbage, cauliflower and capsicum suffered disadvantage of use of an internal standard, less sensitive, lack of selectivity, use of expensive detectors, long time for analysis and tedious procedures. Considering this drawback, there was a need to develop more advantageous methods for its determination in cabbage, cauliflower and capsicum. For this reason, an attempt has been made to develop a simple, sensitive, cost effective and reliable HPLC with UV detection (method M14) for estimation of chlorpyrifos residues in cabbage, cauliflower and capsicum without the use of internal standard.

2.14. CYPERMETHRIN

The use of pesticides in agriculture concern of residue which may affect on human health. Cypermethrin is a relatively toxic and it is used to control moth pests of fruits and vegetable crops. As a consequence cypermethrin residue can be found in fruits and vegetables. Concern over the cypermethrin residues in fruits and vegetables have led to the development of many analysis methods.

described spectrophotometric methods for the estimation of cypermethrin in environmental and biological samples.

Several researchers proposed different methods for the analysis of cypermethrin in vegetables and fruits. HPLC with UV detection (Pang et al., 1995; Mohammed et al., 1997; Jin et al., 2009; Sheheli et al., 2009; Barkat et al., 2012; Shailendra et al., 2012), gas chromatography with electron capture detection (Ramesh and Balasubramanian, 1998; Sannino et al., 2003; Zhang et al., 2006; Zawiyah et al., 2007; Mohammad et al., 2010; Subhash et al., 2010; Shailendra et al., 2012; Mahgoub et al., 2012), gas chromatography with mass spectrometric detection (Ramesh and Balasubramanian, 1998; Sannino et al., 2003; Rosa et al., 2008; Tahir et al., 2011; Shinde et al., 2012; Subhash et al., 2012) and enzyme-linked immunosorbent assay (Eun et al., 2004; Mikaela et al., 2009) methods were reported.

To the best of our knowledge three different methods for quantitative analysis of cypermethrin residues in cabbage, cauliflower and capsicum have been developed; they are HPLC with UV detection (Pang et al., 1995; Sheheli et al., 2009; Barkat et al., 2012; Shailendra et al., 2012), gas chromatography with electron capture detection (Zhang et al., 2006; Zawiyah et al., 2007; Subhash et al., 2010) and gas chromatography with mass spectrometric detection (Subhash et al., 2012). Though the gas chromatography methods are highly sensitive, main disadvantages are the necessity of expensive and sophisticated instrumentation. The HPLC with UV detection methods possess deficiencies such as use of internal standard, lack of sensitive, less precise and less accurate.

Therefore, an attempt has been made in the present study to develop a simple, sensitive, cost effective and reliable HPLC with UV detection (method M15) for estimation of cypermethrin residues in cabbage, cauliflower and capsicum without the use of internal standard.
2.15. OXYTETRACYCLINE

Oxytetracycline is a broad-spectrum antibiotic commonly used in human and veterinary medicine for therapeutic and prophylactic purposes. Oxytetracycline is also used as feed additives in animal husbandry. Their residues were found in several animal products such as milk, eggs, fish or meat. In apiculture beekeepers use oxytetracycline against the bacterial diseases that affects honeybees (Gunes et al., 2009). As a result, oxytetracycline residues can be detected at trace levels in honey of treated bees. Oxytetracycline residues show a relatively long half-life and may show direct toxic effects on consumers (Gunes et al., 2009). Therefore, the presence and maximum residue values of oxytetracycline residues in honey should be regulated.

Several liquid chromatographic with different detection methods have been applied for the analysis of oxytetracycline residues in infant formula (Roya et al., 2011), beef liver (Oka et al., 1985), fish tissues (Nordlander et al., 1987; Liu et al., 2013), cattle meat (Muriuki et al., 2001), surface water (Santiago et al., 2007), chicken muscle (Mulders and Vande, 1989; Liu et al., 2006), porcine muscle (Mulders and Vande, 1989; Walsh et al., 1992; Sokol and Matisova, 1994), bovine muscle (Mulders and Vande, 1989; Walsh et al., 1992; Sokol and Matisova, 1994; Oka et al., 1997), milk (Oka et al., 1994; Long et al., 1995; Zhao et al., 2004; De Ruyck and De Ridder, 2007), animal feeds (Martinez and Shimoda, 1988), catfish (Long et al., 1990), egg (Liu et al., 2013) and shrimp (Liu et al., 2013).

Cháfer et al. (2010) and Yongnian et al. (2011) described enzyme-linked immunosorbent assay and voltammetry methods for the determination of oxytetracycline fish. Chen and Gu (1995) developed capillary electrophoresis method for determination of oxytetracycline levels in cow milk, serum, and urine. An indirect conductimetric screening method was developed for rapid detection of oxytetracycline residues in bovine carcasses by Myllyniemi et al. (2002).
Different analytical methods are reported for the determination of oxytetracycline residues in honey. These include HPLC with UV detection (Galeano et al., 1990; Bonta et al., 2007; Li et al., 2008; Hakuta et al., 2009; Hai et al., 2010; Anna et al., 2013), HPLC with fluorescence detection (Argauer et al., 1991; Pena et al., 2005; Narin and Supaporn, 2008; Supaporn and Narin, 2009; Sun et al., 2009), Liquid chromatography with mass spectrometry (Ishii et al., 2006; Carrasco et al., 2008; Gunes et al., 2009; Jing et al., 2009; Mei et al., 2012), capillary high performance liquid chromatography (Huang et al., 1999), HPTLC (Imdad et al., 2013), high performance capillary electrophoresis (Chen et al., 2001), Immunological assay (Wim et al., 2007), Capillary zone electrophoresis (Casado et al., 2007) and spectrophotometric methods (Wish et al., 2011). The above reported methods suffers from one or more drawbacks such as use of internal standard, expensive detectors, cumbersome procedure, long analysis time, less sensitive, lack of precision and accuracy.

Hence, an attempt has been to develop a simple, sensitive, cost effective and reliable HPLC with UV detection (method M16) for estimation of oxytetracycline residues in honey without the use of internal standard.

2.16. PERMETHRIN

The insecticide, permethrin, has been used in agriculture applications because of their effectiveness in insect control in field crops. The use of permethrin has resulted in occurrence of permethrin residues in water, soil, crops, vegetables and fruits. The consumption of water, crops, vegetables and fruits contaminated with permethrin residues shows suppressive effects on the immune system of humans. Therefore a rapid method for monitoring residue levels of permethrin in above said samples is desirable. Pang et al. (1994), Guomin et al. (2000), Iffat et al. (2007) and Shishovska et al. (2010) reported gas chromatography with electron capture detection, enzyme-linked immunosorbent assay, gas chromatography with electron capture detection and gas
chromatography with electron capture detection methods for the determination of permethrin residues in fruits, river water, wheat and wine samples, respectively.

Gas chromatography with electron capture detection (Pang et al., 1994; Srivastava et al., 2011), with mass spectrometry (Ana et al., 2002), Liquid chromatography with fluorescence detection (Vázquez et al., 2008), with mass spectrometry (Martínez et al., 2006) were described for the analysis of permethrin residues in different vegetable samples. Some reviewed literature describes the HPLC with UV detection (Arayne et al., 2011; Shishovska and Stefova, 2012) and spectrophotometric (Kazemipour et al., 2002) methods for the determination of permethrin in formulations.

Literature survey reveals that only gas chromatography with electrospray mass spectrometry (Ravikumar et al., 2013), with electron capture detection (Pang et al., 1994) were reported for the determination of permethrin residues in paddy. Also, in the reviewed literature HPLC with UV detection method is not reported for the estimation of the permethrin residues in paddy samples. Therefore, in the present study an attempt has been made to develop a simple, sensitive, cost effective and reliable HPLC with UV detection (method M18) for estimation of permethrin residues in paddy with out the use of internal standard.
AIM AND OBJECTIVES

Aim:

To develop and validate simple, cost effective, precise and accurate reverse phase high performance liquid chromatographic methods for the selected antibiotics and pesticides. Application of the developed methods for the analysis of selected antibiotics and pesticides in the selected food stuffs.

Objectives:

1. Development of the method
   i. Selection of mobile phase solvent
   ii. Optimization of the HPLC parameters
   iii. HPLC Performance calculations (Retention time, Theoretical Plates, Plates per Meter, Peak asymmetry)

2. Validation of the method: Determination of the following parameters for the developed methods
   i. System suitability studies
   ii. Selectivity
   iii. Linearity
   iv. Sensitivity
   v. Precision
   vi. Accuracy

3. Extraction of the selected antibiotics and pesticides from the selected food stuffs.

4. Application of the developed and validated methods to the selected food stuffs.