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Nature and Science

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

REVIEW ON ANTIBIOTICS RESIDUE LEVELS IN TISSUES OF SLAUGHTERED SMALL RUMINANTS

Nuru Mohammed Mekonnen

Debre Birhan Sheep Multiplication and Breed Improvement Center, P.O. Box 464, Debre Birhan, Ethiopia.
Email: nurum0686@gmail.com

Abstract: The adverse effects of consuming food products containing antibiotic residues range from gastrointestinal disturbances to more serious risks like teratogenicity and allergic reactions. Moreover, the development of antibiotic-resistant pathogens poses a global health threat, affecting both humans and animals. Strict adherence to withdrawal periods and maximum residue limits, along with judicious use of antibiotics under veterinary supervision, is essential for mitigating these risks and safeguarding public health. Additionally, continued research and monitoring are crucial for identifying emerging threats and adjusting regulations as necessary to ensure food safety and prevent antibiotic resistance.

[Nuru Mohammed Mekonnen. **Review on Antibiotics Residue Levels in Tissues of Slaughtered Small Ruminants.** *Nat Sci* 2024,22(5):12-20]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature 02. doi:10.7537/marsnsj220524.02.

Keywords: Antibiotics, residue, small ruminants

1. Introduction

Oxytetracyclines are broad-spectrum antibiotics because they are active against gram positive and gram negative bacteria. They also act against some pathogenic agents unaffected by other antibiotics e.g., *Rickettsiae*, certain large viruses belonging to Psittacosis group in animals and lymphogranuloma venerum group in humans. Oxytetracyclines have activity against mycoplasmas, spirochetes and actinomycetes. At high doses some antiprotozoan activity has also been observed. Tetracycline undergo metabolism to various degrees. The most frequently identified substance in urine, feces and tissue is the parent tetracycline. As much as 30% is excreted unchanged in feces. Oxytetracyclines are reversibly bound to plasma proteins and are widely distributed. Oxytetracyclines diffuse throughout the body and are found in highest concentrations in kidney, liver, spleen and lungs. They are also deposited at active sites of ossification (Riviere and Spoo, 2001).

Oxytetracycline (OTC) is commonly used in livestock and poultry for prevention and treatment of various diseases. However, the use of this compound may result in residues in animal derived food products, especially if withdrawal times are not observed. These residues may pose a health threat to consumers, depending on the type of food and the amount of residue present. The acceptable Maximum Residue Limit (MRL) for OTC as recommended by the joint FAO/WHO Expert Committee on Food Additives (1999) is 0.2, 0.6 and 1.2 µg g^{-1} for meat, liver and kidney, respectively. OTC residues that exceed the tolerance level may be of toxicological concern. Human health problems resulting from intake of sub-chronic exposure levels of oxytetracycline include gastrointestinal disturbances (Baker and Leyland, 1983), teratogenic risk to the fetus, allergic reactions (Schenk and Collery, 1998) and development of resistant pathogens for human and animals (Senyuva *et al*., 2000). Tetracyclines in meat may potentially stain the teeth of young children (Walton *et al*., 1994). Microbial resistance to antibiotics is a worldwide problem in human and veterinary medicine. It is generally accepted that the main risk factor for the increase in antibiotic resistance is an extensive use of antibiotics. This has led to the emergence and dissemination of resistant bacteria and resistance genes in animals and humans. In both populations antibiotics are used for therapy and prophylaxis of infectious diseases. The main sector of resistance-increasing medicine usage, in regard to human health, lies within the health care sector. The antimicrobial agents used in animal care are also significant, not only in increasing the resistance in animal pathogen, but also in bacteria transmitted from animals to humans (Senyuva *et al*., 2000).

Resistance to members of tetracycline group has been reported for some strains of the following bacteria: Escherichia, Aerobacter, Salmonella choleraesuis, Salmonella typhimurium, Salmonella dublin, Salmonella pullorum, Salmonella gallinarum, Chlamydia psittaci, Proteus, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, Pasturella multocida, Klebsiella pneumoniae and Beta-haemolytic Streptococci (Kunin, 1993). Drug residue remains very significant from the prospective of international trade and consumer confidence, because it results in international trade barrier. Therefore, the aim of this review is to give overview of antibiotics residue levels in tissues of slaughtered small ruminants.

2. World Meat Production, Consumption and Trade

2.1. Production

The total world meat production in 2010 was 294.2 million tones, of which pig meat accounted for 109.3 million tones; poultry 98.9 million tones, bovine 66.7 million tones and 13.7 million tons of Ovine meat. The increase in production is mainly due to strong economic growth and consumption in Asia supporting output gains while an ease in trade barriers prompts an increase in slaughtering and production in South America (FAO, 2012). Looking at different meat producing regions and countries Asian countries are the leading producers of all meat types accounting for 26, 32, 59, 61, and 43 percent, respectively of bovine, poultry, pig, ovine and all meats. Of all the countries, China is the world number one producer of pig and ovine meat. It has a share of 51% for pig; 34% for ovine and 30% of the total meat production of the world. The United States of America is the leading producer of bovine and poultry meat accounting for respectively for 18 and 23 percent of the world output (FAO, 2012).

The African continent with the production level of 12.7 million tons of all meat types, accounts for about 4.5 percent of the world meat production. Of the African countries, South Africa is the leading meat producer. Algeria, Nigeria, South Africa and Sudan account for 45 percent of the ovine meat production of the continent (FAO, 2012).

Sheep and goat meat markets steady as increased supplies prompt a decline of prices. Ovine meat markets have recovered from two years of declining supplies, with global output estimated up one percent to 13.9 million tons in 2012. Satisfactory pasture conditions have induced flock rebuilding throughout many of the major producing areas of Asia and Africa, including major producers as Pakistan, the Islamic Republic of Iran, India and Turkey. Meanwhile, sheep meat production in the developed countries, which accounts for only 22 percent of global supplies, is forecast to increase only slightly, with lower output in Europe and the North America offset by a 4 percent increase in Australia and New Zealand which have benefited from favorable pasture conditions and high lamb crops (FAO, 2012).

2.2. Trade

Over all the total world meat trade in 2010 were 26.7 billion (US $), of which pig meat accounted for 6.2 billion (US $); poultry 11.7 billion (US $), bovine 7.7 billion (US $) and 0.8 billion (US $) of Ovine meat. A recovery in global supplies, combined by booming shipments to China and strong demand in many Middle Eastern markets are supporting a 2 percent rise in sheep and goat meat trade in 2012 to 757,000 tones. While imports by the EU, the United States and Canada are slipping as a result of the faltering consumer demand, imports by Middle Eastern countries, in particular Kuwait and Saudi Arabia are expected to keep rising, despite a sustained trade in live sheep and goats coming from Ethiopia, Sudan and Somalia (FAO, 2012).

2.3. Consumption

A recent study made by the World watch Institute's Nourishing the Planet project, has found that worldwide per-capita meat consumption increased from 41.3 kg in 2009 to 41.9 kg in 2010 – about half a kilo more per year. Taking all factors into account, including people living on the edge of starvation in the developing world, compared to those living in the industrial world, there is a big discrepancy between the 32 kg per year average for people living in the developing world and an average of 80 kg for those in the industrial world (FAO, 2012). Countries fill the supply gap through importing different meat types from other countries. South America where intensive commercial beef production is practiced; North America, US being the leading beef producer, Oceania (Australia and New Zealand) are the net exporting countries while the rest including Asia, Africa, central America and Europe are net importers of meat. Except in ovine meat in which it is self-sufficient, the gap between demand and meat production gap in Africa is met through importation (FAO, 2012).

3. Use of Antimicrobials for food animals and their impact

3.1. Therapy

Therapy is the administration of antibiotics to animals or groups of animals in order to treat animal illness. When farm animals exhibit clinical diseases a common method of treatment is the use of appropriate antibiotics. Veterinary medical advances made it possible to locate, treat, and prevent catastrophic animal health risks that could be detrimental to the agriculture sector as a whole (Saridaki-Papakonstadinou *et al.*, 2006). For many farm animal diseases and illness prove to be readily amenable to antibiotic immunizations. At the first signs of animal distress, the owner or farmer will refer the case to animal physician, veterinarian, who will then discern the course of antibiotic treatment, is necessary. The main objective in employing antibiotic therapy treatment in farm animals is to limit the progression of disease in a population of animals. Also the therapy use of antibiotics is meant to treat the specific animal's illness because disease negatively impacts and decreases farm animals' performance and abilities. The principal goal of the antibiotic agents, in a therapy situation, is to treat the animals' infections and destroy the harm causing pathogens as quickly as possible while also causing minimal adverse effects to the animal in question (Vellicer, 2004). In doing so, the specific antibiotic will bind to the target site, or active site, of the organism to attack the disease causing pathogens. Therapeutic applications of antibiotics in farm animals are essential components to increase profitable and humane livestock production in response to broad economic pressure of consumer demand for protein. Therapeutic uses of antibiotics must be under the advice and discretion of a practicing animal physician. Farmers, ranchers, and farm animal owners alike all must consult the advice of a veterinarian prior to the employment of antibiotic treatment to sick animals (Barragry, 1994). Similarly, the full course of antibiotic treatment for farm animals requires a valid prescription and the oversight of a veterinarian. This being said, malpractice and misuse of antibiotics on farm animals, i.e. using therapeutic antibiotics to treat disease in farm animals without a prescription or without a veterinarian's opinion, inevitably does occur. In addition, farmers, ranchers, and animal owners must only use antibiotics approved by relevant national and international authorities, which monitor antibiotic use for farm animals to ensure not only the effectiveness in treatment but also safety for the animals and their meat and milk products (Dibner and Richards, 2005). As stated in the American Dairy Association and Dairy Council's review in 2003, "All milk is strictly tested for antibiotics on the farm and at the processing plant. Any milk that tests positive [for antibiotics] cannot be sold to the public" ensuring both meat and milk are rigorously monitored for the presence of antibiotics. Overall, therapeutic antibiotic use in animal agriculture results in healthier animals, which in turn provides for healthier sources of food for humans. Therefore, in terms of antibiotic use for farm animals, the health promotion benefits should be highlighted despite the controversial effects of antibiotic use for animal growth promotion (Ortelli *et al.*, 2004).

3.2. The use of antimicrobials as performance enhancers

The earliest evidence of the growth promoting effects of antibiotics became apparent in chickens exposed to small doses of chlortetracycline which grew more rapidly than non-exposed chickens. Tetracyclines are considered to have a negative impact on

the commensal microflora of the intestine and therefore their use as performance

enhancers is not recommended. In poultry, performance enhancers, such as

bacitracin and virginiamycin can also control *Clostridium perfringens* infections, which

are potentially fatal. Estimates suggest that the average benefit of such products is

an improvement in feed conversion rate (FCR) of approximately 3%, with a range of

0-5 % (Bedford, 2000). The mechanism of action of antibiotics as growth promoters is

related to interactions between the antibiotic and the gut microbiota, thus the direct

effects of antibiotic growth promoters on the microflora can be used to explain

decreased competition for nutrients and reduction in microbial metabolites that

depress growth (Dibner and Richards, 2005).

3.3. Health impacts of Antibiotics

Antibiotics used in food animals can affect the public health because of their secretion in edible animal tissues in trace amounts usually called residues. For example, oxytetracycline (Saridaki-Papakonstadinou *et al.*, 2006) and Chloramphenicol residues (Ortelli *et al.*, 2004) have been found above the regulatory standards in honey. Some drugs have the potential to produce toxic reactions in consumers directly while some others are able to produce allergic or hypersensitivity reactions (Vellicer, 2004). For example, β-lactam antibiotics can cause cutaneous eruptions, dermatitis, gastro-intestinal symptoms and anaphylaxis at very low

doses. Such drugs include the penicillin and cephalosporin groups of antibiotics (Paige *et al.*, 1997). Indirect and long term hazards include microbiological effects, carcinogenicity, reproductive effects and teratogenicity. Microbiological effects are one of the major health hazards in human beings. Antibiotic residues consumed along with edible tissues like milk, meat, eggs and honey can produce resistance in bacterial populations in the consumers. These bacteria might then cause difficult-to-treat human infections. Certain drugs like 3-nitrofurans and nitroimidiazoles can cause cancer in human population. Similarly, some drugs can produce reproductive and teratogenic effects at very low doses consumed for a prolonged period of time.

4. Oxytetracycline

4.1. Structure and solubility

Oxytetracycline (OTC) is one of the oldest antibiotics still in use in medicine. It is a yellow amphoteric crystalline compound with a molecular weight of 460.44. It has both a low solubility in water and a low octanol/water partition coefficient. It is stable as a powder but unstable in solution and therefore injections of oxytetracycline are often formulated as hydrochlorides (Treves-Brown, 2000).

4.2. Mechanism of activity

Oxytetracycline possesses antimicrobial activity by binding to the 30S ribosomal subunit of susceptible organisms. Upon binding, the oxytetracycline interferes with transfer RNA's ability to bind with messenger RNA, thereby preventing bacterial protein synthesis (Riviere and Spoo, 2001).

4.3. Toxicity, distribution and absorption

Oxytetracycline has a low order of toxicity and a high ability to readily disperse into blood and most tissues (Kapusnik-Uner *et al*., 1996). However despite these merits, oxytetracycline has a rather limited bioavailability (the total amount of the drug absorbed) because it chelates or forms complexes with polyvalent cations such as Ca^{++}, Fe^{++}, Al^{+++}, and Mg^{++} (Riviere and Spoo, 2001). These electrically charged complexes, which are microbiologically inert, are not able to easily traverse the lipid-rich biological membranes thereby causing a several fold decrease in the absorption of oxytetracycline (Riviere and Spoo, 2001; Treves-Brown, 2000). In addition, oxytetracycline also will form complexes with organic material and clay.

4.4. Chronic health effects Oxytetracycline

Oxytetracycline (OTC) is a broad-spectrum antibiotic used to treat a variety of infections and is also used as a growth promoter in animals. Symptoms of chronic exposure to oxytetracycline include blood changes (leucocytosis, atypical lymphocytes, lung congestion, toxic granulation of granulocytes and thrombocytopenia purpura) (Stolker and Brinkman, 2005). Liver injury and delayed blood coagulation may also occur. It can damage calcium rich organs such as teeth and bones and sometimes causes nasal cavities to erode. Children under 7 years of age may develop a brown discoloration of the teeth. Infants of mothers treated with OTC during pregnancy may develop discoloration of the teeth. Some other chronic effects of oxytetracycline include increased sensitivity to the sun, wheezing and asthmatic attack. Toxicological studies indicate that this drug is not mutagenic, carcinogenic, or terratogenic (Stolker and Brinkman, 2005).

5. Methods for detection of antibiotic residues

5.1. High performance liquid chromatography (HPLC)

High performance liquid chromatography is a technique where substances in a mixture are separated by passing the mixture through a packed column with a solid stationary phase. The substances separate from each other because they interact differently with the solvent that is passed through the column, as well as the mobile phase and the surface of the stationary phase based on their chemical properties. The substances that have weak interactions with the stationary phase will travel faster through the column than the ones that have stronger interactions (Lindsay, 1992). Depending on the structure of the stationary phase and the composition of the mobile phase different separation mechanisms can be achieved. Ion exchange, absorption and size exclusion are a few different examples of separation mechanisms (Gréen 2007).

a. Development of HPLC

High Performance Liquid Chromatography (HPLC) is a chromatographic technique of great versatility and analytical power (Reuhs and Rounds, 2010). The technique shares a similar mechanism with other liquid chromatography techniques where different components of the sample move through the column with moving solvent at different speeds and become separated due to difference in molecular size or weight, solubility, and affinity to the column packing

material. HPLC represents the modern culmination of classic liquid chromatography (Snyder *et al*., 2009).

A basic HPLC system consists of a pump, injector, column, detector, and data processing system. In a HPLC system, the solvent is continuously pumped through the column, and the separated sample components are continuously sensed by a detector when they leave the column (Reuhs and Rounds, 2010). This technique was developed in the mid-1970s and quickly improved with the development of column packing materials and the additional convenience of on-line detectors. In the late 1970's, new methods including reverse phase liquid chromatography allowed for improved separation between very similar compounds.

b. Mobile phase reservoir

The solvent competes with the analytes for binding sites on the stationary phase. The greater the elution strength of the solvent, the more easily it displaces the analyte. In reversed-phase chromatography the stationary phase used is non-polar or weakly polar while the mobile phase is more polar. The less polar a solvent is, the higher its elution strength becomes. The mobile phase can consist of buffers, organic solvents or water that can be used separately or in a mixture (Gréen, 2007; Harris, 2003). There are two different ways for elution. Either isocratic elution, by using the same composition of mobile phase, during the whole run or a gradient elution, where multiple solvents are used and where their ratios are modified continually, during the analysis. Gradient elution is used to increase the elution strength so that more strongly retained analytes elute in a reasonable amount of time (Harris, 2003). If the sample is dissolved in a solvent that has much greater elution strength than the mobile phase, double peaks or altered retention times might occur. To solve this problem the sample should be dissolved in a solvent that has lower elution strength, or in the mobile phase itself (Harris, 2003).

c. Pump

A pump is used to deliver the mobile phase through the column at high pressure with a controlled flow rate. It is important that the flow is non-pulsing or else it would affect the chromatogram resulting in fluctuations of the baseline. It is necessary to remove dissolved air or air bubbles from the mobile phase before it reaches the pump, or else it could cause variations in the pressure by irregular pumping actions or even stop the pump from working at all. The use of either a degasser in line with the pump that the mobile phase is passed through, or by soliciting the mobile phase before connecting the container to the system, solves the problem (Lindsay, 1992).

d. Injection system

The injector consists of a valve with an injection loop. A sample is transferred from a syringe at atmospheric pressure into the loop. This can be done either manually or automatically, using an auto sampler. When the valve is turned from load to inject position the loop becomes connected with the mobile phase delivery system and the sample is transferred into the column (Gréen 2007, Lindsay 1992).

e. Column

The most common dimensions of HPLC columns are 5-30 cm in length with an inner diameter of 1-5 mm. The packing material that is most frequently used in HPLC columns is particles of silica. These are small and porous with spherical or asymmetrical shape and they normally have a diameter of 3-10 µm. To protect the column from impurities that can be present in the sample or the solvent a filter or a short guard column, that contains the same stationary phase as the main column, is often used in front of the column (Harris, 2003). The silanol (Si-OH) groups of the silica particles in the column can be chemically modified, where additional groups are attached that alters the properties of the silica surface. When working with analytical HPLC the stationary phase often consists of modified silica, i.e. a bonded phase, where the attached chemical groups provide different retention. The most commonly used is the non-polar C18 column (Lindsay, 1992). In modern columns the silica has sometimes been replaced by polymers with wider pH stability.

f. Detection

An ideal detector should be specific for the analytes, sensitive enough to detect low concentrations of the analytes, providing a linear response and not broadening the eluted peaks (Harris, 2003). The detection methods used during this thesis work were; UV detector, photodiode array detector and an ion-trap mass spectrometer.

6. Validation of an analytical method

The applicability of analytical methods is assessed by a validation process. Validation is the formal and systematic way to demonstrate the suitability of a developed method for testing the analyte to provide useful analytical data within defined limits (Maldener, 1989). Method validation studies comprise

the overall procedure established during method development including sample preparation, analysis and the assessment of the results. The applicability and the requirements mainly depend on the analyte being tested, the analytical method used and the area of application of the method. The most common validation parameters for the analytical methods are discussed below.

6.1. Selectivity and specificity

Selectivity is the ability to separate the target analyte from interferences present in the sample. It is considered the most important parameter in the analytical method validation to provide accurate analyte measurements. Commonly, the term selectivity is used interchangeably with the term specificity. Actually, when the method is poorly selective, a serious mistake is made by describing it as specific. Therefore, one should distinguish between these two terms. Selectivity refers to the ability of the method that can produce responses for a number of analytes in the complex matrix and discriminate the response of a single analyte from the other (Vessman, 1996). Whilst, specificity describes the method that produces a response for only one single analyte. The International Union of Pure and Applied Chemistry (IUPAC) recommended the promotion of the selectivity concept and settled the problem by expressing the idea that "specificity is the ultimate of selectivity" (Vessman *et al*., 2001). Analytical chemists in chromatography, therefore, should use these two terms carefully and selectivity should be given top priority in all analytical method developments (Aboul-Enein, 2000). Usually, ith using UV detection in HPLC analysis the term of selectivity is very common since it can detect many components present in a sample. The selectivity should be tested against all components present in the sample matrix by using a blank sample with and without the analytes. These components or interferences have to be separated with acceptable resolution (Rs > 1.5) (Maldener, 1989).

6.2 Accuracy and precision

The accuracy of an analytical method refers to the closeness of the measured value obtained to the true value. Practically, no measurement process is ideal, therefore, the true or actual value cannot be exactly known in any particular measurement. Certified reference materials (CRMs) can be used to assess the accuracy of the measurements determining the difference between the measured value and the true value and then to estimate the size of the actual error. CRMs are "materials or substances, one or more of whose property values are certified by a procedure that establishes traceability to an accurate realization of the unit in which the property values are expressed and for which each certified value is accompanied by an uncertainty at a stated level of confidence" (King and Grp, 2003). Quantitatively, the accuracy and the precision are essential to assess the associated errors in the analytical method (Manoli and Samara, 1999). Thus, it is crucial to identify the sources of errors affecting the accuracy and subsequently to find a better procedure to remove and reduce the impact of these errors. Experimental errors are classified into three major types; gross, systematic and random errors. Gross errors are defined as the errors causing damage to the experiment and require a new experiment. Systematic errors are that the same errors remain constant for the measurement repeated under the same conditions. The term bias is used to describe a systematic error. Normally, the bias of a measuring instrument can be calculated by the observed value that is described as being biased positive or negative when a systematic error is present. Some sources of systematic errors include spectral interferences and standard preparation. Random errors can be defined as the errors which vary randomly when replicate measurements are carried out under identical conditions. This type of error is inescapable and requires the utmost of care to minimize. Random errors affect the precision, whereas both random and systematic errors influence the accuracy. Another parameter incorporating random and systematic errors is called uncertainly. It is commonly used to describe a realistic range within which the true value of the quantity being measured is expected to lie (Miller and Miller, 2005). Sample preparation is the main parameter affecting uncertainty measurement (Meyer and Majors, 2002).

The precision of an analytical method is defined as the degree of an agreement among individual tests obtained when the method is applied to multiple sampling of a homogenous sample. It is usually expressed in terms of standard deviation (SD) or relative standard deviation (RSD %) for more than five replicate measurements of the standard at low, mid and high concentrations. Four types of precision can be characterized.

Repeatability (instrument precision) is evaluated by repeated measurement of the same sample to test the efficiency of the instruments. All instrumental measurements produce some random error or noise which is difficult to remove. However it can be evaluated by suitability testing. The second type is repeatability (intra-assay precision) which is assessed by repeating sample analysis in one laboratory by one analyst using the same conditions. The third type is intermediate precision obtained using the same laboratory and analytical procedure under different

operating conditions. Lastly, the most important type of precision is reproducibility when analyzing the same solution under different conditions including different laboratories, analysts and instruments (Green, 1996).

6.3 Linearity and range

The linearity of an analytical method refers to the ability to obtain results either directly, or after mathematical transformation proportional to the concentration of the analyte in the sample within a given range (Shabir, 2003; Chandran and Singh, 2007). Linearity is established by measuring the instrument response of a sufficient number (at least five) of standard solutions in the expected range of the analyte. It is estimated by the equation of the regression line (y = ax + b) by plotting concentrations (x) versus the response (y) (Caldas *et al*., 2009). Some distributed errors are expected to be associated with the regression line. The error source from the measured response is more than the error in the preparation of a sample concentration. Typically, the correlation coefficient is used to express the acceptability of the linearity of the regression line (Chandran and Singh, 2007). However, according to different views in the literature, there is a problem in the terminology used for linearity criteria. Authors refer to five different expressions for the linearity criteria including r, r^2, and correlation coefficient, correlation coefficient with r and correlation coefficient with r^2. Statistically, the Pearson Product-Moment Correlation Coefficient (PMCC) is typically denoted as r. In the case of a straight line graph, the value r^2 is the same as the coefficient of determination and denoted as R^2 which is calculated from the regression line of the calibration curve provided R^2 as a decimal by Excel, but is given as percentages if multiplied by 100. The value of r^2 is always slightly smaller than r (Miller and Miller, 2005). The coefficient of determination R^2 (r^2) explains the variation from the regression line as a percentage. The total variability is expressed by the variability that can be explained from the regression line and the remaining variability is due to other unexplained factors. For example if r^2 is 89% that means 89% of the variability of the response of y from the regression line can be explained and 11% of the remaining variability is unexplained.

Linearity criteria are reported as a mixture of correlation coefficient of r and coefficient of determination R^2 (r^2) values. Typically, a correlation coefficient of more than 0.995 is considered acceptable for the analysis of biological samples for HPLC assay. For an HPLC method of pharmaceutical samples at low levels the correlation coefficient should be $\geq$ 0.98 (Green, 1996). The linearity specification for autosampler performance is acceptable when r^2 is 0.998 or more (Hall and Dolan, 2002; Shabir *et al*., 2007). Under most conditions, the correlation coefficients according to Chandran and Singh (2007) should be greater than 0.9999. In analytical practice, calibration curves with correlation coefficient r values greater than 0.99 are relatively common (Miller and Miller, 2005). The range of the method is the interval between the upper and lower levels of an analyte in the sample with acceptable accuracy, linearity and precision (Shabir, 2003; Chandran and Singh, 2007). The range is estimated on either a linear or nonlinear response curve, using the data of the linearity studies and the intended application of the method (Green, 1996).

Misinterpretation of the determination of the range can be avoided by plotting the concentration or (log concentration) either against the deviation from the regression line or against the ratio of response to concentration. A nonlinear calibration may be required in a specific analytical method but mostly a linear type is chosen (Chandran and Singh, 2007). In chromatographic measurements, three calibration curve methods are used to quantify the analyte accurately: standard addition, external standard and internal standard methods (Wieling *et al*., 1992). The standard addition method is practically suitable to samples with an analyte concentration close to the sensitivity limit to solve the matrix effect problem.

The drawback of this method is that each sample must be analysed many times and it is suitable for measuring only a small number of samples. In the external standard method, a compound present in pure solution is analyzed separately from an unknown sample under the same conditions. However, this method has some disadvantages, because each step must be controlled regularly (Wieling *et al*., 1992). The use of the internal standard method is very common to achieve precise results in environmental applications. It can decrease the contribution of systematic errors to the total errors of the determination (Ostroukhova and Zenkevich, 2006). The internal standard can be added before sample pre-treatment to improve the reproducibility of the analytical method thus eliminating the variance of the injection volume in the chromatographic system. In addition, it can correct for any losses of the analyte during sample preparation. In some cases, adding the internal standard can be done immediately before the chromatographic analysis rather than before sample preparation but this can only correct for analyte loss associated with the chromatographic measurements not the entire of the analysis procedure (Ostroukhova and Zenkevich, 2006).

The internal standard should be selected to mimic the analyte, thus important criteria are considered when choosing the internal standard such as

its peak completely resolved, same chemical properties as the target analyte, its retention time close to that of the analyte, detectable under the same conditions as the analyte and absent from the original samples. However, using an internal standard may be associated with some interference that may cause some measurement errors. Therefore, to achieve the best results, the analytical method should be examined with and without an internal standard in order to ensure its suitability for use (Wieling *et al*., 1992; Aboul-Enein, 1998).

7. Conclusion and recommendation

Antibiotic residues in food products pose a serious threat not only to public health but also to international trade and consumer confidence. Residues can lead to antibiotic resistance, rendering these crucial medications ineffective in treating bacterial infections in both humans and animals. Strict legislation and regulations are essential to prevent the unauthorized and illegal distribution and use of antibiotics in the veterinary field. Requiring prescriptions from veterinarians helps ensure that antibiotics are used appropriately and responsibly, minimizing the risk of residues and promoting the judicious use of these drugs. This approach is crucial for safeguarding public health, maintaining food safety standards, and facilitating smooth international trade without unnecessary barriers.

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4/22/2024