

Qualitative Immunoassay Approach for The Determination of Tetracyclines Antibiotics Residues in Milk Samples Followed by a Quantitative Improved HPLC-DAD method: The Case of Saudi Market

Moneera N. Alnassrallah*, Nourah Z. Alzoman, Aliyah A Almomen

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, King Saud University

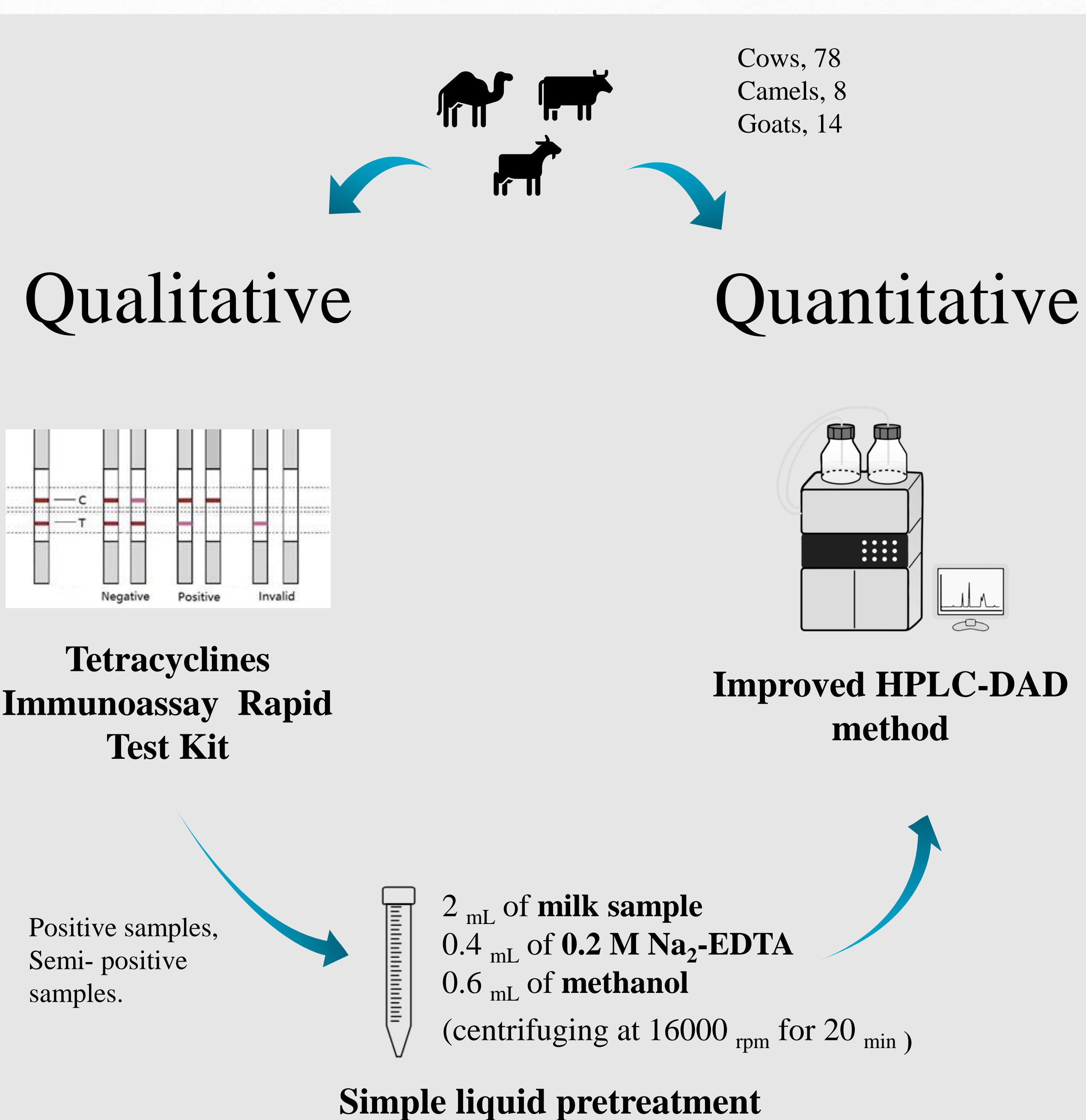
INTRODUCTION

Recently, there has been a global renewed interest in restricting antibiotics prescriptions due to the emerging of multidrug-resistant bacteria for human treatment, however, the other part of the story is that bacteria may develop resistance in many hosts, such as plants, animals and soil, and can adversely affect human health thereafter. Prior researches have demonstrated that the use of high levels of antibiotics to increase animal productivity is associated with the presence of their residues in animal-based food. Tetracyclines antibiotics are among the most used antibiotics in animal farming due to their effectiveness and low cost. Previous studies and review articles have reported their presence in milk samples around the world. Specifically, in the case of Saudi Arabia it is seldom studied, and it is unclear to what extent these antibiotics have residues in milk samples^{1,2}.

OBJECTIVES

- Improve an HPLC-DAD method in term of resolving power and sensitivity.
- Simplify the extraction procedure.
- Implement a qualitative approach for the detection.
- Investigate milk samples from the Saudi market.

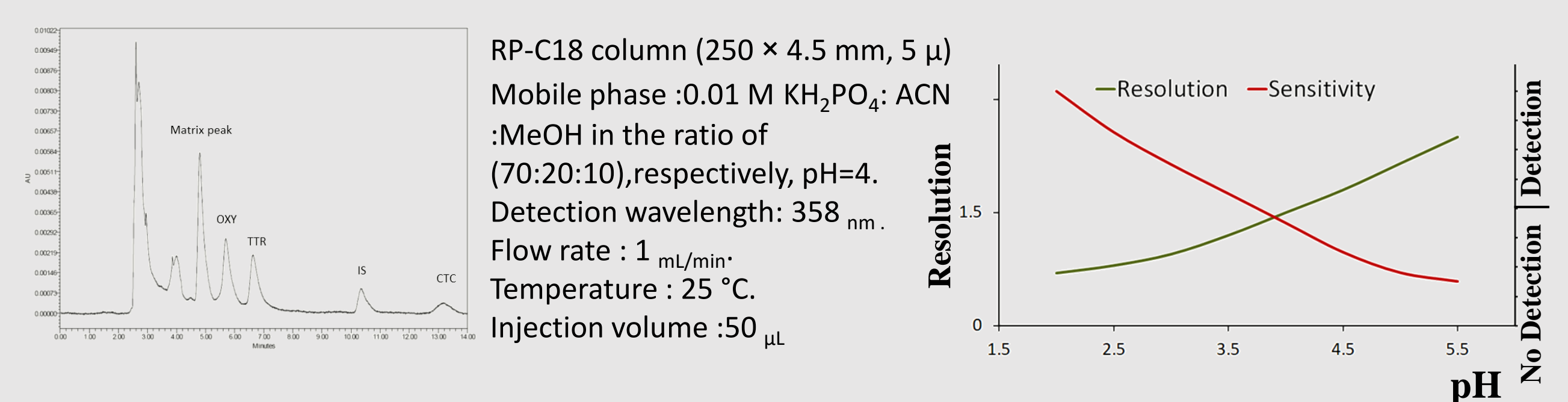
METHODS



RESULTS & DISCUSSION

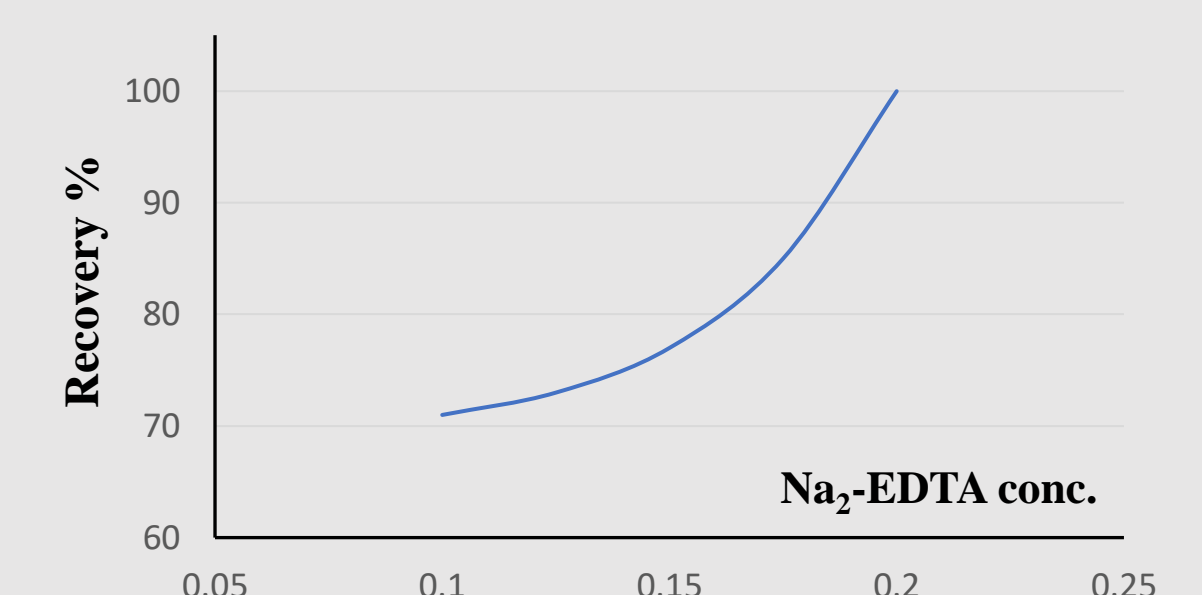
• Optimization of the HPLC-DAD

Many HPLC-DAD methods are reported for the separation of TCs antibiotics in milk matrix, however some degree of peak interference was observed between the matrix peak and the Oxytetracycline peak^{3,4}. Incorporating and optimizing these conditions have led to an improved HPLC-DAD method that has a satisfactory resolution in milk matrix.



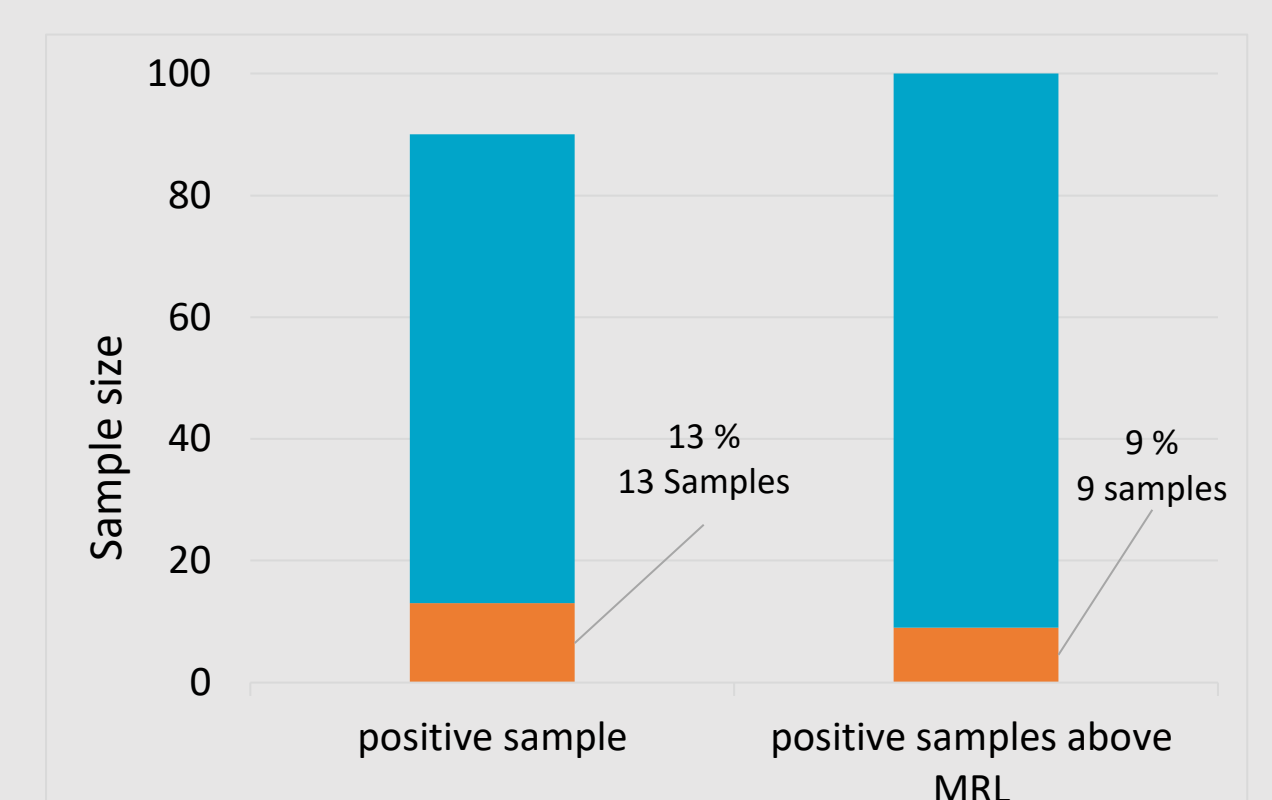
• Liquid Extraction

This pretreatment procedure was simplified to be effective in term of recovery percentage (Recovery 85-100%), facility of the work and time saving. Two key factors were proportionally related to the recovery %: 1) conc. of Na-EDTA, and 2) centrifugation force (time × speed).



• Analysis of milk samples

Sample size was 100 milk samples from three animals' species (cows, camels and goats). The next bar chart show their analysis results. The stated maximum residual limits (MRL) for tetracyclines antibiotics is 100 ng/mL. Tetracycline antibiotic was the most detected antibiotic, followed by Oxytetracycline. Chlortetracycline residues have never been detected due to its limited use in animal feeding.



CONCLUSION

- Regarding the proposed method, it is validated according to the guideline of the International Conference on Harmonization (ICH).
- Its evidently clear from the finding that the occurrence of TCs antibiotics residues in milk products is below the Maximum Residual limit (MRL), which reflects a good clinical practice in the use of those antibiotics in animal feeding and treatment.

REFERENCES

- 1) Darwish, W. S.; Eldaly, E. A.; El-Abbasy, M. T. et al. 2013. Antibiotic Residues in Food: The African Scenario. *Jpn. J. Vet. Res.* vol 61; p S13. DOI: 10.14943/jivr.61.suppl.s13
- 2) Sabhya Sachi; Jannatul Ferdous; Mahmudul Hasan Sikder; S M Azizul Karim Hussani. Antibiotic Residues in Milk: Past, Present, and Future. *Journal of Advanced Veterinary and Animal Research* 2019, 6 (3), 315–332.
- 3) Saridal, K.; Ulusoy, H. I. A Simple Methodology Based on Cloud Point Extraction Prior to HPLC-PDA Analysis for Tetracycline Residues in Food Samples. *MICROCHEMICAL JOURNAL* 2019, 150.
- 4) Saleh, H.; Elhenawee, M.; Hussien, E. M.; Ahmed, N.; Ibrahim, A. E. Validation of HPLC-UV Multi-Residue Method for the Simultaneous Determination of Tetracycline, Oxytetracycline, Spiramycin and Neospiramycin in Raw Milk. *Food Anal. Methods* 2020.