ISBN 978-989-9121-13-3
31st ISTANBUL International Conference on "Chemical, Agriculture, Biological and Environmental Sciences" (ICBEN-22)
Nov. 23-25, 2022 Istanbul (Turkey)

Detection and Identification of Tetracycline Residues in Milk By High Performance Liquid Chromatography (HPLC) and ELISA: -Comparative Study Between The Two Methods-

Latifa Boultif * [1], Bassem Chebira [1], Mohamed Cherif[1]Abdeldjelil, Assia Boudebza [1]

[1]PADESCA laboratory, Institute of Veterinary Science, University Mentouri of Constantine, Algeria.

Abstract: *The presence of antibiotic residues, particularly in milk, is a subject of concern, due to their known harmful effects: on human health (antimicrobial resistance, allergic problems, etc.); on dairy industry (economic losses); and during bacteriological tests (false negative results). The objective of this study is to identify and quantify oxytetracycline residues in milk, using two analytical methods: ELISA and HPLC, then to compare the two methods.120 milk samples are analyzed by an indirect competitive ELISA. To quantify residues, a calibration curve is established using standards,. Positive samples revealed by ELISA are then analyzed by HPLC, whose analysis parameters and extraction method have been fixed previously.Oxytetracycline concentrations obtained by the two methods are compared using Student's t-test.The ELISA analysis reveals 36 samples containing tetracyclines at concentration levels of 5 to 74 µg / L. While only 22 samples containing oxytetracycline were revealed by HPLC at concentrations levels of 7,74 to 76,24 µg / L. Statistical comparison between concentrations revealed by the two analytical methods, shows no significant difference (p-value of 0,01 and 0,05). However, when samples are taken one by one; in most cases (68%), the values provided by HPLC analysis are slightly higher than those obtained by ELISA. HPLC is an extremely sensitive method which can quantify antimicrobial residues at the nanogram scale. On the other hand, the ELISA assay is a specific method because it allows to characterize the molecule to be assayed. ELISA is also less expensive than HPLC, but visibly of diminished sensitivity.*

Key words*: Tetracycline, Antibiotics Residues, Milk, ELISA, HPLC.*

1. Introduction and objective:

The presence of antibiotic residues, particularly in milk, is a subject of concern, due to their known harmful effects: on human health (antimicrobial resistance, allergic problems, etc.) (Châtaigner and Stevens, 2005; Leroy and Fanir, 2005; Sanders, 2005; Guy et al., 2004) on dairy industry (economic losses); and during bacteriological tests (false negative results) (Form, 2003; Brouillet, 2002; Maghuin-Rogister et al., 2001). Tetracycline is one of the most widely used molecules in rural livestock production, particularly for the treatment of respiratory, genital and podal diseases (Van Boeckela et al., 2015; Desalegne, 2011; Sarmah et al., 2006; Furusawa, 2003). It is also a molecule commonly excreted in milk (Zahid Hosen et al., 2010). It is thus, very likely to be found, as antibiotic residues in milk, one of the foods, of animal origin, the most consumed in Algeria, because it represents the main source of protein of animal origin.

The objective of this study is to identify and quantify oxytetracycline residues in milk, using two analytical methods: ELISA and HPLC, then to compare the two methods.

2. Material and methods

120 milk samples are analysed by an indirect competitive ELISA. To quantify residues, a calibration curve is established using standards,. Positive samples revealed by ELISA are then analysed by HPLC, whose analysis parameters and extraction method have been fixed previously.

Oxytetracycline concentrations obtained by the two methods are compared using Student's t-test.

3. Results and Discussion

The ELISA analysis reveals 36 samples containing tetracyclines at concentration levels of 5 to 74 µg / L. While only 22 samples containing oxytetracycline were revealed by HPLC at concentrations levels of 7,74 to 76,24 µg / L (table n°1). Statistical comparison between concentrations revealed by the two analytical methods, shows no significant difference (p-value of 0,01 and 0,05). However, when samples are taken one by one; in most cases (68%), the values provided by HPLC analysis are slightly higher than those obtained by ELISA.

TABLE I: comparison of the values obtained by HPLC analysis and those obtained by ELISA for the quantification of oxytetracycline (µg/l)

échantillons	HPLC	ELISA	Moyenne	Ecart-type	variance
A6	25,42	22	23,71	2,41	5,84
A8	22,25	20	21,125	1,59	2,53
H2	26,74	25	25,87	1,23	1,51
H3	45,98	42	43,99	2,81	7,92
J3	7,74	5,3	6,52	1,725	2,97
J8	64,34	65	64,67	0,46	0,21
J10	14,72	18	16,36	2,31	5,37
D8	22,07	15	18,53	4,99	24,99
E1	42,09	45	43,54	2,05	4,23
E5	29,86	25	27,43	3,43	11,80
E6	52,09	46	49,04	4,30	18,54
F1	35,83	30	32,915	4,12	16,99
F2	20,97	22	21,48	0,72	0,53
F4	44,13	40	42,06	2,92	8,52
F6	35,44	40	37,72	3,22	10,39
F7	50,86	52	51,43	0,80	0,64
F8	62,53	60	61,26	1,78	3,20
F9	15,85	12	13,92	2,72	7,41
G5	17,76	18	17,88	0,16	0,02
G6	76,24	74	75,12	1,58	2,50
G9	31,46	30	30,73	1,03	1,06
G10	17,44	15	16,22	1,72	2,97
t = 0,74					

We noticed a similarity between our working method and that of other researchers. Thus in 2012, Kamberi and Sulaj first used ELISA as a qualitative detection method, then HPLC for the quantification

of oxytetracycline. Out of 161 samples, 5 are positive, and only 2 (1.4%) exceed the MRL at values equal to 290 µg/L and 1600 µg/L.

4. Conclusion

HPLC is an extremely sensitive method which can quantify antimicrobial residues at the nanogram scale. On the other hand, the ELISA assay is a specific method because it allows to characterize the molecule to be assayed. ELISA is also less expensive than HPLC, but visibly of diminished sensitivity.

5. References:

[1] Brouillet P (2002). Résidus de médicaments dans le lait et tests de détection. Bulletin des GVT. 15: 2541.

[2] Châtaigner B, Stevens A (2005). Investigation sur la présence de résidus d'antibiotiques dans les viandes commercialisées à Dakar. Institut Pasteur of Dakar. Pp 6-9.

[3] Desalegne, A. S. (2011): Detection and determination of oxytetracycline and penicillin G antibiotic residue levels in bovine bulk milk from debrezeit and nazareth dairy farms. Proceedings of the 1st International Technology, Education and Environment Conference (c) African Society for Scientific Research (ASSR).

[4] Form G (2003). Les résidus inhibiteurs dans le lait. Evolution des méthodes de détection Facteurs de risques en région Rhône-Alpes. These of veterinary medecin. University Claude-Bernard-Lyon. Pp 2630.

[5] Furusawa N (2003). Isolation of tetracyclines in milk using a solid-phase extracting column and water eluent. Talanta. 59(1): 155-159.
https://doi.org/10.1016/S0039-9140(02)00472-1

[6] Guy PA, Royer D, Mottier P, Gremaud E, Perisset A, Stadler RH (2004). Quantitative determination of chloramphenicol in milk powders by isotope dilution liquid chromatography coupled to tandem mass spectrometry. J. Chromatogr. A. 1054(1-2): 365-371.
https://doi.org/10.1016/S0021-9673(04)01290-7

[7] Leroy P, Fanir F (2005). Méthode statistique en médecine vétérinaire. Veterinary Faculty of Liege. Pp 49-133.

[8] Maghuin-Rogister G, Janosi A, Helbo V, Van Peteghem C, Sander E, Van Eeckhout E, Cornelis M, Jouret M (2001). Stratégie intégrée d'analyse qualitative et quantitative des résidus de substances antimicrobiennes dans les denrées alimentaires. Services scientifiques du premier Ministre Affaires scientifiques, techniques et culturelles (SSTC) . France. Rapport Final SSTC. Pp 13-58.

[9] Sarmah, a. K. Meyer, m. T. Boxall, a. B. (2006): A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics in the environment. Chemosphere. 65, 725–759.
https://doi.org/10.1016/j.chemosphere.2006.03.026

[10] Sanders P (2005). L'antibiorésistance en médecine vétérinaire: enjeux de santé publique et de santé animale. Review: Bulletin de l'académie vétérinaire de France. 158(2): 139-140.
https://doi.org/10.4267/2042/47761

[11] Van boeckela, t. Brower, c. Gilbert, m. Grenfell, b. T. Levin, s. A. Robinson, t. P. Teillant, A. Laxminarayan, R. (2015): Global trends in antimicrobial use in food animals. PANS 2015.112(18), 5649–5654.
https://doi.org/10.1073/pnas.1503141112

[12] Zahid, hosen, s. M. Mostafa kamal, a. T. M. Sabuj barua, m. Sheikh anwar, m. D. Kishor, m. Hassan kawsar, m. D. Arifuzzaman, m. D. (2010): Detection of Residual Antibiotics in Fresh Cow Milk. Bangladesh Pharmaceutical Journal; Vol. 13. 2, 64-66.