

Validation for simultaneous determination of tetracycline by using QuEChERS method and LC-MS/MS in liver of buffalo

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Abstract: The aim of this study is to determine a method validation for the presence of tetracycline antibiotics (Tetracycline (TC), Oxytetracycline (OTC), Chlortetracycline (CTC) and Doxycycline (DOC) in liver tissue of buffalo. LC-MS/MS was used for determination of tetracycline antibiotic residues. Separation was carried out by with electrospray ionization (ESI) in positive -ion mode using mobile phase at a flow rate of 400 ml/min and injection volume 5 ul. The recoveries of analyzed drugs were in between 75 and 80 ppb

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Key words: Tetracyclines, LC-MS/MS, validation, EU commission.

1. Introduction

Tetracyclines (TCs) are essential group of antibiotics which were used in farm animals mass production and in veterinary medicines. They were commonly used for the prevention and treatment of dairy cattle for several infectious diseases ,Tetracyclines could be administrated orally through food or drinking water(Alfredsson, et al 2008),However ,TCs were given to animals for human consumption , not only to treat and stop some diseases , but also to promote growth, the rich and wrong use of TCs may result in the presence of their residues in edible animal tissues , which might be toxic and risky for human health , and might lead to serious allergic reactions , Moreover the accumulation of TCs might lead to the evolution of microorganisms frustrating resistance to antibiotics . The European Union residue tolerance for DOC, OTC, TC, and CTC is 100 ppb in tissue which is globally confirmed (Samanidou, et al 2009)

TC's were problematic to analyze because they are unstable, and dut to their ability to bind with proteins, so, it is important to take these considerations into account when developing a method or when doing routine analysis of these antibiotics. Most reported methods for TC analysis were time-consuming and its sample preparation procedures are weak sample cleanup. As a result, there was a need for a simple method for the analysis of TC antibiotics in liver tissue of buffalo. Liquid-liquid extraction/partitioning was difficult to perform due to TC's charge and low affinity for organic solvents. Therefore, solid-phase extraction (SPE) combined with LC-MS/MS analysis was used for the determination of TC residues in liver tissue (Lopez, 2014)

The method was based on liquid-liquid extraction followed by liquid chromatography-tandem mass spectrometry (LC-MS) determination with electrospray ion source (ESI) in positive

mode. The method was simple, fast and inexpensive for simultaneous analysis of selected antibiotics which are common in use with liver tissue. (Zhu, 2009)

Liver samples were vortexed and homogenized in mixture of EDTA-Citrate buffer solution at PH 4.0 and acetonitrile followed by centrifugation. Repeated extraction with acetonitrile was performed the supernatant was combined and evaporated till dryness. Additional acetonitrile volume was added to the sample to facilitate the evaporation process reaching to complete dryness and the residue was re-dissolved in methanol/buffer (25:75). Quantitation and confirmation of each compound was done by liquid chromatography tandem mass spectrometry (LC-MS/MS) with electrospray ionization (ESI) in positive -ion mode, the mass spectrometer was operated in multiple reactions monitoring (MRM) mode. Two different MRM were used for confirmation.

2. Methods and materials

Chemicals and materials

Methanol and acetonitrile were HPLC graded and were purchased from Merck (Darmstadt, Germany). Sodium hydroxide, citric acid monohydrate, ammonium hydroxide, and formic acid were purchased from Riedel-deHaen ≥99%. Ethylenediamine Tetra Acetic Acid disodium salt dihydrate (Na₂-EDTA) was purchased from Fluka ≥99%. Commercial antibiotic (SAs) standard was supplied by (Dr.Ehrenstorfer, Germany).

Extraction

2.0g ± 0.1 (W) sample liver of buffalo was weighed in disposable 50 ml plastic with screw cap tubes with Laboratory balance, capable of weighing to 0.1 mg ,then it was fortified with the four concentrations 25 ul,50 ul,100 ul and 200 ul of DOC, OTC, TC, and CTC each one with six

replicates, after that 1 ml from both 1 M Sodium citrate buffer at PH 4 and 0.5 Na₂EDTA were added on the tubes, moreover 10.0 ml of Acetonitrile will be added then were homogenized for 2-3 min using Homogenizer, Ultra-Turrax IKA @T25digital. And then washed for one minute. Followed by the centrifugation at 4000 rpm for 10 min at 4 °C using Centrifuge, BIOFUGE Primo R; THERMO) then the supernatant was decanted into 100 ml round-bottomed flask. The extraction was repeated with another 10 ml Acetonitrile and shaken for 1 min followed by centrifugation again as previously described and combined the supernatant at the same 100 ml round-bottomed flask. After that the flasks were evaporated using Rotary evaporator (Heidolph VV2000) at 35±2 °C. However aqueous bubbles must be avoided in order to avoid the back-suction. Then 2 ml of Methanol buffer was re-dissolved into the flasks then sonicated using Sonicator. After that the samples were filtered using disposable acrodisc 0.45 µm coupled with 5 ml plastic syringe in amber vial and will only 15 µl of the sample were injected into Liquid chromatography tandem mass spectrometry (LC-MS/MS) equipped with electrospray ion source API 4000 QTrap. The instructions were followed which mentioned in the instrument log

book for LC-MS/MS operation and conditions.

3. Results and Discussion

Complete validation of TCs was done according to EU decision 37/2010 the MRL of TCs 300 µg/kg, and all parameters were complied with EU requirements, trueness, coefficient variation (CV) and Recovery were calculated at three different concentrations according to EU decision i.e. at 25ppb, 50ppb and 100ppb of MRL. However an extra concentration of 200ppb was calculated in order to increase the accuracy and linearity

Linearity and sensitivity

Commonly, the quantification of antibiotic residues was performed by using a matrix-matched calibration curve made from fortified blank samples prepared in the same matrix as the real samples. To test the linearity of the calibration curve, four standards of TCs in the blank liver matrix were analyzed. The calibration curve showed a good linearity, for the calibration curve based on pure standard solutions prepared in methanol, indicating that there was a matrix enhancement showed in Figure 1

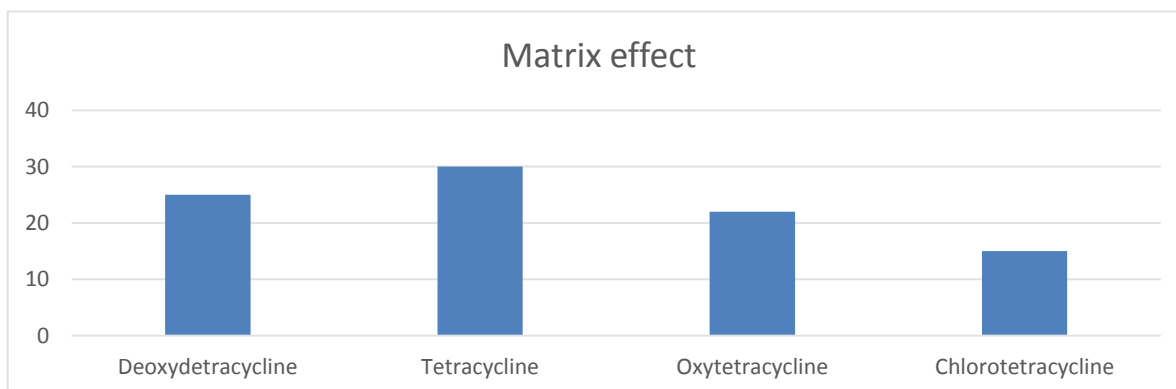


Figure 1: matrix effect of TCs from liver samples

Recovery

The recoveries were obtained by spiking blank pool samples at four different levels (25, 50, 100 and 200 µg kg⁻¹). Recoveries for fortified samples were reported in Table 1. Overall recoveries of OTC ranged from (73 to 75 µg kg⁻¹)

, recoveries of CTC ranged from (73.25 to 78.5 µg kg⁻¹), recoveries of DOC ranged from (73.7 to 77.8 µg kg⁻¹) while recoveries of TC ranged from 74.2 to 78.3 µg kg⁻¹, meeting Commission of the European Guidelines for RSD 3.9-12.8%

Table 1: Mean and overall recoveries of TCs from liver samples fortified at (25, 50, 100 and 200 µg kg⁻¹).

Overall Relative Standard Deviation (RSD %)				Average Recoveries %				Analytes
200 (µg/kg)	100 (µg/kg)	50 (µg/kg)	25 (µg/kg)	200 (µg/kg)	100 (µg/kg)	50 (µg/kg)	25 (µg/kg)	
3.9	4.7	7.6	12.7	74.1	73.7	77.8	75	OTC
4.4	4.8	6.5	14.6	73.26	73.25	78.5	77.6	CTC
3.9	4.7	7.6	12.7	74.4	73.7	77.8	75	DOC
4.3	4.9	7.0	12.8	74.2	74.4	78.3	78	TC

Repeatability and Reproducibility:

Forty eight samples of the blank pool were fortified with the analytes to yield concentrations of 25, 50, 100 and 200 $\mu\text{g kg}^{-1}$ ($n = 6$, each level). This procedure was repeated on two days with the same aseptic conditions. Overall inter laboratory relative repeatability (RSDr) and overall within-

laboratory reproducibility (RSDR) for all residues at 25, 50, 100 and 200 $\mu\text{g kg}^{-1}$ were found below 15% (Table 2), meeting Codex Alimentarius and Commission of the European Communities guidelines for repeatability (20 and 15 %,) respectively, when the target level is $>10 \mu\text{g/kg}$.

Table 2: Repeatability and Reproducibility for the determination of TCs in spiked liver samples

Overall Relative Standard Deviation (RSD %)			
200 ($\mu\text{g/kg}$)	100 ($\mu\text{g/kg}$)	50 ($\mu\text{g/kg}$)	25 ($\mu\text{g/kg}$)
3.9	4.7	7.6	12.7
4.4	4.8	6.5	14.6
3.9	4.7	7.6	12.7
4.3	4.9	7.0	12.8

Decision limits ($CC\alpha$) and Detection capabilities ($CC\beta$):

The EU decision introduces the concepts of decision limit ($CC\alpha$) and detection capability ($CC\beta$) for a chemical analytical method. These parameters are to be used instead of the more familiar limit of detection and limit of quantification. The definition of the $CC\alpha$ for a forbidden compound is: "The limit at and above which it can be concluded with an error probability of 1% that a sample is noncompliant". The

definition of the $CC\beta$ for a forbidden compound is: "The lowest concentration at which a method is able to detect truly contaminated samples with an error probability of 5%". $CC\alpha$ and $CC\beta$ were calculated at level 100 ppb in matrix of liver. For $CC\alpha$ and β calculation following equations was used. As shown in table 3

$CC\alpha = \text{MRL} + 1.64 * \text{SD}$ of 20 fortified blanks at MRL

$CC\beta = CC\alpha + 1.64 * \text{SD}$ of 20 fortified blanks at $CC\alpha$

Table 3: Calculation of $CC\alpha$ and $CC\beta$ of TCs by fortification at the level 100 ppb

$CC\beta$	$CC\alpha$	Analytes
112.156	105.729	OTC
112.122	105.772	CTC
112.343	105.729	DOC
112.144	106.034	TC

References

- 1- Alfredsson, G., Branzell, C., Granelli, K., & Lundström, Å. (2005). Simple and rapid screening and confirmation of tetracyclines in honey and egg by a dipstick test and LC-MS/MS. *Analytica Chimica Acta*, 529(1), 47-51.
- 2- Andersen, W. C., Roybal, J. E., Gonzales, S. A., Turnipseed, S. B., Pfenning, A. P., & Kuck, L. R. (2005). Determination of tetracycline residues in shrimp and whole milk using liquid chromatography with ultraviolet detection and residue confirmation by mass spectrometry. *Analytica Chimica Acta*, 529(1), 145-150.
- 3- Andreu, V., Vazquez-Roig, P., Blasco, C., & Picó, Y. (2009). Determination of tetracycline residues in soil by pressurized liquid extraction and liquid chromatography tandem mass spectrometry. *Analytical and bioanalytical chemistry*, 394(5), 1329-1339.
- 4- Blasco, C., Di Corcia, A., & Picó, Y. (2009). Determination of tetracyclines in multi-specie animal tissues by pressurized liquid extraction and liquid chromatography-tandem mass spectrometry. *Food Chemistry*, 116(4), 1005-1012.
- 5- Cháfer-Pericás, C., Maquieira, Á., Puchades, R., Miralles, J., & Moreno, A. (2013). Multiresidue determination of antibiotics in feed and fish samples for food safety evaluation. Comparison of immunoassay vs LC-MS-MS. *Food Control*, 22(6), 993-999.
- 6- EUROPEAN COMMISSION REGULATION (2010) NO 37/2010 of 26 January 2010 on aying down requirements on the quality of aeronautical data and aeronautical information for the single European sky, *Official Journal of the European Union* 15/19 P.9-10
- 7- Lopez, M. I., Pettis, J. S., Smith, I. B., & Chu, P. S. (2014). Multiclass determination and confirmation of antibiotic residues in honey using LC-MS/MS. *Journal of agricultural and food chemistry*, 56(5), 1553-1559.
- 8- Nakazawa, H., Ino, S., Kato, K., Watanabe,

- T., Ito, Y., & Oka, H. (1999). Simultaneous determination of residual tetracyclines in foods by high-performance liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry. *Journal of Chromatography B: Biomedical Sciences and Applications*, 732(1), 55-64.
- 9- Samanidou, V. F., Nikolaidou, K. I., & Papadoyannis, I. N. (2009). Development and validation of an HPLC confirmatory method for the determination of seven tetracycline antibiotics residues in milk according to the European Union Decision 2002/657/EC. *Journal of separation science*, 30(15), 2430-2439.
- 10- Yang, S., Cha, J., & Carlson, K. (2004). Quantitative determination of trace concentrations of tetracycline and sulfonamide antibiotics in surface water using solid-phase extraction and liquid chromatography/ion trap tandem mass spectrometry. *Rapid communications in mass spectrometry*, 18(18), 2131-2145.
- 11- Zhu, J., Snow, D. D., Cassada, D. A., Monson, S. J., & Spalding, R. F. (2009). Analysis of oxytetracycline, tetracycline, and chlortetracycline in water using solid-phase extraction and liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A*, 928(2), 177-186.