[volume 41 | supplement 1 | june 2018]

JOURNAL OF Veterinary Pharmacology and Therapeutics

Including veterinary toxicology

14th International Congress of the European Association for Veterinary Pharmacology and Toxicology held in Wroclaw, Poland, June 24–27, 2018

Guest edited by Błażej Poźniak, Marcin Świtała and Johanna Fink-Gremmels

ISSN 0140-7783 (paper)
ISSN 1365-2885 (online)

Available online at wileyonlinelibrary.com/journal/jvp

WILEY

Discover this journal online at
Wiley Online Library
wileyonlinelibrary.com/journal/jvp

J O U R N A L O F
Veterinary Pharmacology and Therapeutics

Including veterinary toxicology

[volume 41 | supplement 1 | june 2018]

14th International Congress of the European Association for Veterinary Pharmacology and Toxicology held in Wroclaw, Poland, June 24–27, 2018

Guest edited by Błażej Poźniak, Marcin Świtała and Johanna Fink-Gremmels

by Real Time PCR, were prepared by serial gravimetric dilutions of ddPCR verified plasmid material, following JRC guidelines for plasmid reference materials (6).

Results and Conclusions: Major validation parameters were checked: LOD (17.92 copies, RSDr% = 27%), LOQ (38.68 copies RSDr% = 21%), PCR efficiency (101.2%), linearity (R^2 = 0.99), accuracy and robustness. Compared to original PgR assay (4), the increased performance of analytical method allowed the reliable quantification of PgR also in difficult samples like RNA extracted from FFPE. The routine application of the present molecular test represents a useful tool to implement biological based monitoring plan to prevent estrogenic illicit treatment and to protect consumers.

References: 1. De Maria R *et al.* (2010) *Vet Rec*; 167: 291–296.
2. Divari S *et al.* (2011) *Food Addit Contam Part A*; 28: 1677–1686.
3. Botta M *et al.* (2016) *Tox Lett*; 258: S280.
4. Uslenghi F *et al.* (2013) *Food Addit Contam Part A*; 30: 253–263.
5. Dong L *et al.* (2014) *Anal Bioanal Chem*; 406: 1701–1712.
6. Cobisier *et al.* (2013) EU Publications.

P9.5 | Illicit treatment in beef cattle: can a stress oxidative marker give a perspective?

M. Pezzolato; R. Barbero; S. Bergagna; S. Gennero; D. Dezzutto; K. Varello; E. Bozzetta

Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy

Introduction: The identification of biomarkers for the detection of illicit anabolic treatment of bovines is necessary to develop new screening strategies to prevent unconscious intake of hormone residues by consumers. The oxidant chemical species are a group of molecules able to remove one or more reducing equivalents. In all living organisms there is a delicate balance between production and elimination of these compounds. In blood, the defense against free radicals is provided by the antioxidant barrier power (PAO), moreover the reactive oxygen metabolites (ROMs) are considered valid biomarkers for oxidative stress in both humans and animals. The aim of this study is to assess whether it is possible to use a combination of oxidative stress indicators as biomarkers of chronic stress occurring during illicit treatments.

Material and Methods: 47 male veal calves were housed for seven months. In the sixth month, animals were divided in three groups: Group A (n = 20) received 19-nortestosterone (50 mg); Group B (n = 20) received a cocktail of 19-nortestosterone (50 mg) and 17 b-estradiol valerate (5 mg) once a week for four weeks, Group K (n = 7) was kept as a control. Blood samples were collected before, during and after the treatment period and PAO and ROMs were analyzed. The antioxidant barrier power was evaluated by measuring the ability to oppose the massive oxidant action of hypochlorous acid. The reactive oxygen metabolites in samples were photometrically quantified based on their ability to generate alkoxyl and peroxyl radicals in the presence of iron, according to the Fenton's reaction. The procedures were carried out according to indications of OXY Adsorbent & d-ROMs Test- Diacron srl and the tests were performed in triplicate.

Results: A decrease of the PAO values and increase of ROMs values were identified. The difference between both treatment groups compared to the control group resulted statistically significant. The decrease of the PAO values and increased levels of ROMs are a signal of oxidative stress, and are correlated with the degree of impairment of antioxidant mechanisms (1).

Discussion: Results showed that the ROMs and PAO could be used as biomarker-based approach to highlight bovine anabolic illegal treatments. Finally, the oxidative stress biomarkers can play a key role in the animal welfare assessment through the detection of stress level to which the animals are subjected.

Reference: 1. Gironi M *et al.* (2014) *Eur J Inflamm*; 12: 351–363.

P9.6 | Development and validation of an analytical technique to quantify amitraz in a PVC strip formulation used in *Varroa* mite control in honey bees

D. Reinaud[1]; V. Perez[1]; P. Vidondo[2]; M. Del Hoyo[2]; C. Lanusse[1]; L. Alvarez[1]; L. Moreno[1]

[1]*Laboratorio de Farmacología, Centro de Investigación Veterinaria de Tandil (CIVETAN), UNCPBA-CICPBA-CONICET, Facultad de Ciencias Veterinarias, Campus Universitario, (7000) Tandil, Argentina;* [2]*Laboratorio de medicamentos veterinarios APILAB S.R.L, Pringles, Argentina*

Introduction/Objective: Amitraz (AMT) is a formamidine ectoparasiticide used in veterinary medicine to control ticks, lice and mites. It is available for topical use as spray, dip, or pour on in dogs, swine and cattle. Argentina is one of the major honey bee producer countries. The mite *Varroa destructor* is a pathogenic parasite, which seriously affect honey production by *Apis mellifera*. It feeds on the haemolymph of brood and adult honey bees, contributing to the spread of viral and bacterial infections. AMT has been approved to use as a sustained-released strip to control varroasis. The goal of the present work was to develop and validate an HPLC analytical methodology to quantify AMT after its recovery from PVC fortified strips (AMIVAR 500®, APILAB S.R.L.).

Materials and Methods: AMT quantification was performed by HPLC with UV detection (288 nm). An Agilent Extend-C18 reversed-phase column (4.6 × 250 mm, 5 μm) fitted with a guard column (4.6 × 12.5 mm, 5 μm) was used for separation. Elution was carried out at an isocratic flow (1 ml per min) using a mobile phase composed by 20 mM triethylamine: acetonitrile (10:90). AMT was extracted from PVC strip samples with methylene chloride for 18 h at 20 ± 2°C. The method underwent an exhaustive validation process following internationally recognized standards (CVMP, VICH, 1998). Considering the purpose of the developed analytical method, the following validation parameters were determined: specificity, linearity, range, precision (repeatability, intermediate precision) and recovery.

Results and Conclusions: The AMT retention time was a 7.5 min. No interferences from endogenous compounds of the PVC strip sample were observed. The ratio *"AMT standard signal/blank noise signal"* was much higher than that required to determine the measurable concentration range (20–30 μg ml^{-1}). The calibration parameters achieved the established acceptance criteria, repeatability of the triplicates (intraday repeatability) with a CV ≤ 2% and correlation coefficient ≥ 0.99. The repeatability (CV) and intermediate precision were ≤ 2% or ≤ 4%, respectively. The recovery was 104% so it was proposed not to correct for recovery in the routine analysis. The obtained validation parameters confirm the linearity of the model, offering a good fit for the proposed working range. Thus, the developed analytical methodology complies with the validation criteria required for AMT quantification in PVC strips. The analytical data shown here is a step forward to assure the correct use of AMT to control *Varroa* mite in honey bee production.

P9.7 | Determination of exposure to topical drug residues upon petting following repeated topical application of a mirtazapine transdermal ointment in cats

O. Szkuta[1]; M. P. O'Banion[2]; M. Vila[1]; M. Cantiello[1]

[1]*Avogadro LS, Fontenilles, France and Terre Haute, IN, USA;* [2]*Kindred Biosciences, Inc, Burlingame, CA, USA*

Introduction/Objective: The user safety of topically administered veterinary medicinal products must be evaluated for marketing authorizations. The objective of this study was to determine the human exposure to mirtazapine residues that could be dislodged from the fur of cats by petting the animal body and ear after repeated applications of a mirtazapine transdermal ointment applied topically.

Materials and Methods: Eight female, domestic short-hair cats, weighing 3.0–4.5 kg, received 2 mg of mirtazapine once daily for 14 consecutive days as topical ointment applied to the inner (anterior) surface of one pinna. On Day 14, cats were stroked using standardized procedure on the treated ear and on the body with cotton gloved mannequin hands before application of the last daily dose and 0.5, 1, 2, 4, 8, 12, 24, 48, 96 h after dosing. Mirtazapine residues were extracted from the gloves using a methanol bath and quantified by a validated HPLC-MS/MS method with a LLOQ of 250 ng per glove.

Results and Conclusions: The results of this study demonstrated that mirtazapine residues obtained by ear petting at 0.5 h (after the last application), 1 and 2 h were approximately 20%, 2.4%, and 1.2% of the daily dose, respectively. Mirtazapine residues obtained by body petting at 0.5 h were 1.0% of the daily administered dose. In conclusion, user exposure can be considered low by petting the animal body. Higher exposure can occur when the ear (application site) is petted within 0.5 h after the application but is reduced by 1 and 2 h.

P9.8 | Development, validation and application to real samples of a liquid chromatography – tandem mass spectrometry method for determination of tetracyclines in beeswax

A. Gajda; M. Antczak; K. Mitrowska; A. Posyniak

Department of Pharmacology and Toxicology, National Veterinary Research Institute, Pulawy, Poland

Introduction: Beeswax is one of the most valuable honeybee products in addition to honey. It finds important application in food, cosmetic and pharmaceutical industry. Recently, beeswax is widely studied and used for human medicine thanks to its antimicrobial or therapeutic properties (effectiveness in the treatment of burns, bruises and inflammation). However, some substances use in apiculture, like tetracyclines can be delivered to hives causing a contamination of honey and honeybee products, as beeswax. Tetracyclines show effectiveness in combating the devastating effects of American foulbrood and European foulbrood, which are infectious bacterial diseases, severely decrease the honey bee population. However, the use of tetracyclines in beekeeping is forbidden by the European Union law, because there are no Maximum Residue Limits (MRLs) for these antibiotics in honey. The analysis of tetracyclines in beeswax and the method development for their determination is particularly important subject, because European Food Safety Authority, as well as RASFF have been reported many incidents of honey samples contaminated with tetracyclines, so there is a high probability of accumulation of these compounds in beeswax.

Material and Methods: For the determination of tetracyclines including oxytetracycline, 4-epioxytetracycline, tetracycline, 4-epitetracycline, chlortetracycline, 4-epichlorotetracycline and doxycycline in beeswax, a liquid chromatography – tandem mass spectrometry (LC-MS/MS) method was developed. The presented method involved melting and dilution of beeswax in *n*-hexane, liquid-liquid extraction with oxalic acid followed by clean-up with solid – phase extraction using weak cation exchange phase. Chromatographic separation was achieved on Luna C18 analytical column using mobile phase consisting of 0.1% formic acid in acetonitrile and 0.1% formic acid in water, within a total run time of 5 min. The overall recoveries were in the range from 75.6% to 115%. The procedure was satisfactory sensitive with the limit of quantification – 2 μg kg^{-1}.

Results and Conclusion: The application of this method was evaluated by the analysis of real beeswax samples. The presence of OTC was confirmed in 5 out of 48 tested beeswax samples, with following OTC concentrations: 2.25, 2.73, 2.86, 12.48, 17.20 μg kg^{-1}, which show that the developed method can be successfully used to determine the level of tetracycline antibiotics in beeswax.

Acknowledgement: The project was funded by the National Science Centre allocated on the basis of the decision number DEC- 2015/19/B/NZ7/02356.