Drug Testing and Analysis

Published online in Wiley Online Library: 14 September 2010

(www.drugtestinganalysis.com) DOI 10.1002/dta.164

The state of the art of residue analysis: the 6th VDRA symposium 2010

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Since the onset of residue analysis (*ca* 40 years ago) a lot of attention has been paid to the amelioration of analytical methods, for example, lowering the limits of detection (LOD) and limits of quantification (LOQ) or decision limits (CCα) and detection capabilities (CCβ), including an increase in the number of analytes, shortening runtimes, increasing sample throughput, amongst others. The state of the art in residue analysis, which was presented at the VDRA 2010 symposium (Hormone and Veterinary Drug Residue Analysis) in Ghent is reviewed in this article. From an analytical point of view, the use of ultra high performance liquid chromatography (UHPLC) hyphenated with accurate mass spectrometry is often used in combination with other (biological) detection systems and 'omic' approaches. Through these techniques more xenobiotic substances turn out to be naturally occurring in some matrices and/or circumstances (e.g. thiouracil, chloramphenicol and semicarbazide).

Keywords: VDRA symposium; residue analysis; state of the art

Introduction

The *International Symposium on Hormone and Veterinary Drug Residue Analysis* (VDRA) started in 1988 as the *International Symposium on Doping and Hormone Residue Analysis*. The name and scope changed in 1992. The 6th VDRA edition, directed by Prof. C. van Peteghem (Ghent University, Belgium), has become one of the leading European meetings for scientific and instrumental advances in residue analysis. As a result of a fair arrangement with the organizing committee of EuroResidue, another important conference in the field, VDRA and EuroResidue meetings have been organized alternatively every two years since1994. The VDRA consists of plenary lectures, presented by invited speakers outstanding in the domain, contributed papers, and a technical exhibition hosted by companies presenting their latest instruments and related products. The 6th VDRA was held in the new Ufo (University Forum) of Ghent University. In this article the state of the art in residue analysis anno 2010, as brought forward during this symposium, is described.

Invited and Oral Lectures

This overview of the symposium's invited and oral presentations is divided into different subject classes with, if possible, some recent references to the author or subject. Most of the papers (oral presentations and posters) will be published in a special issue of *Analytica Chimica Acta*.

Analytical techniques and new approaches

The subject of high resolution screening of residues and contaminants by liquid chromatography mass spectrometry (LC-MS) was handled by P. Furst (Münster, Germany). P. Furst is one of the top specialists in operating heavy MS instruments for the detection of contaminants such as dioxins.[1] In this talk, especially the use of Orbitrap instruments, such as the Thermo Scientific Exactive for screening purposes was highlighted. Also A. Kaufmann (Zürich, Switzerland) handled the possibilities and limitations of current UHPLC-Orbitrap technologies for multiresidue purposes. A special item in this context was the comparison between tandem MS and high resolution accurate mass MS as a detection system.[2] T. Bovee (RIKILT, Wageningen, the Netherlands) on the other hand discussed the added value of bioassays for the screening of unknown compounds with a special emphasis on yeast estrogen and androgen assays (amongst others, the detection of Diethylstilbestrol (DES) in a food supplement).[3] Omic approaches as a possible future in new screening strategies for illegal growth-promoters in breeding animals were presented by G. Pinel (Laberca, Nantes, France). The speaker highlighted that 'until now' targeted approaches only allow to 'find what we are looking for'. With omic approaches, untargeted profiling and effect-based measurements may also be carried out as new screening tools.[4] M. Mooney (Belfast, UK) also presented effect-based omic approaches to detect illegal growth-promoter use in food-producing animals. In light of the BIOCOP project[5] several animals were treated with different substances. By using protein, metabolite, and blood chemistry profiling, discrimination between treated and control animals could be performed by principal component analysis. M. Bremer (Wageningen, the Netherlands) presented a promising approach for the simultaneous detection of rBST-related biomarkers in serum of cattle by multiplex flow

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cytometric immunoassays. By following 3 biomarkers *ca* 96 samples can be analyzed within 3.5 h. However, the false negative rate of this method is still too high.[6] The influence of anabolic combinations of an androgen and an estrogen on the biochemical pathways in bovine ovary was handled by C. Becker (Munich, Germany). By using the gene expression pattern, discrimination between treated and untreated animals could be made.[7] Finally, the identification of anabolic steroids in herbal- and sports-supplement preparations using bioassay-guided fractionation, UPLC-ToF-MS analysis, and accurate mass database searching was reported by R. Peters (Wageningen, the Netherlands).[8]

Validation of methods

The validation of analytical methods for veterinary drug residue control on an internationally recognized format was handled by E. Verdon (CRL, Fougères, France). The speaker stated that validation, in combination with quality assurance, is the pillar of analytical performance in a worldwide trading market. He also referred to the new, recently published guidelines for validation of screening methods.[9] P. Jedziniak (Pulawy, Poland)[10] elaborated on one important element of validation, namely the evaluation of matrix effects, especially the reduction of ionization suppression in multiclass residue LC-MS methods. Validation of residues of antibiotics in milk was covered by two oral contributions. W. Reybroeck (Melle, Belgium) described the validation of Eclipse 50 and Delvotest accelerator for screening inhibitors in milk. These results were expressed in spider web graphs providing a clear overview of the method performances.[11] The characterization and validation of a new microarray analysis platform for parallel and rapid detection of various antibiotics in raw milk was described by K. Kloth (Munich, Germany). The speaker stressed that by direct injection of milk and automated image processing, the method is applicable for routine circumstances.[12]

Group A and Annex IV substances: (semi-)endogenous status

J. Scarth (Fordham, UK) elaborated on the detection of endogenous steroid abuse in meat production. Possible approaches for developing and applying appropriate detection methods for large animal population studies in the UK were presented. Additionally, he proposed maximum levels for several hormones in various animal species.[13] B. Le Bizec (Nantes, France) discussed nandrolone (nortestosterone) as an example of a molecule with a long history as a semi-endogenous hormone, in animal breeding as well as in sports doping. During his presentation, he provided evidence that estranediol steroid profiles may serve as new criteria to retrieve nandrolone abuse in bovines.[14]

J. Vanden Bussche (Merelbeke, Belgium) presented a new U-HPLC-MS/MS method for the analysis of the xenobiotic thyreostat, thiouracil (TU) without derivatization.[15] This method confirmed the endogenous presence of thiouracil in the urine of several animal species[16]. Moreover, the presence of thiouracil in urine of domesticated animals (the dog) and even humans was demonstrated for the first time. L. Stolker (Wageningen, the Netherlands) provided evidence for the natural occurrence of the banned antibiotic chloramphenicol (CAP) in herbs and grass. This forbidden Annex IV substance was detected in several grass specimens sampled in Mongolia and also in herbs commercially available in the Netherlands.[17] The origin of this contamination is, however, still unknown. The occurrence of semicarbazide (SEM)[18] in meat and shell of Bangladeshi freshwater shrimp was discussed by G. Kennedy (Belfast, UK). Until now, several batches of tiger prawns were refused as a consequence of this positive analysis for SEM. The speaker discussed the possible contamination of prawn meat by the high prevalent natural levels in the shell and the possible pathways of formation (amongst others, an amino acid with a similar structure to SEM).

Veterinary drugs

In a plenary lecture, V. Curtui (EFSA, Parma, Italy) elaborated on the ongoing activities of EFSA[19] and its data collection activities in the field of veterinary medicinal product residues. The collected data, which have been derived from the different member states, are, however, inconsistent. Therefore, EFSA is working on a harmonization of these data in order to create a lucid database containing all individual test results. The simultaneous identification and quantification of five groups of veterinary drugs by HPLC-MS/MS was discussed by C. Nebot (Lugo, Spain).[20] In addition, a number of contributions dealt with coccidiostats: during the first session, the problem of residues of coccidiostats in the poultry sector was addressed in an invited lecture. E. Daeseleire and S. Croubels, both former co-workers of the Bromatology laboratory, described in a joint talk the withdrawal time of veterinary drugs in practice (marker residue depletion studies) and the possible use of a mathematical model to predict residue levels in biological matrices.[21] During the next session, the EU legislation on unavoidable carryover of coccidiostats into non-target feed and resulting levels in food of animal origin, was treated by F. Verstraete (Directorate General for Health and Consumers, European Commission, Brussels, Belgium).[22] The speaker underlined that the aim of this legislation was to keep the contamination level of coccidiostats as low as possible (ALARA: as low as reasonably achievable). The problem of coccidiostats was also addressed in two other oral presentations originating from the CONffiDENCE and RESPOUL projects, respectively.[23,24] U. Vincent (Geel, Belgium) talked about analytical challenges in cross-contamination of coccidiostats from target feed to non-target feed (a multiplex immunoassay, amongst others). The residue levels of veterinary drugs in products of poultry origin as a result of cross-contamination in poultry feed was handled by V. Vandenberge (Melle, Belgium).

R. J. Fussell (York, UK) explained the importance of honey as a food product. He presented a study on the distribution of veterinary drug residues in treated bee hives and the implications thereof for setting maximum residue limits (MRLs); he especially highlighted the fate of ciprofloxacin and inter-hive variation.[25] Finally, the depletion of amantadine (an antiviral drug) in poultry tissues was presented by D. Chan (York, UK). He claimed a rapid depletion from muscle and egg samples and also the necessity of monitoring the parent component.[26]

Pharmaceuticals and the environment

With regards to the interaction between pharmaceuticals and the environment A. Boxall (York, UK) posed the following rudimental question: Do pharmaceuticals have environmental side effects? The answer to this question was obviously affirmative and illustrated with examples such as the *Gammarus Pulex* (common freshwater shrimp) and the water boatman (*Notonectidea*). He also illustrated the fate and impact of diclofenac in vultures. [27]

The occurrence of pharmaceuticals, perfluorinated compounds, and pesticides in biota in the Belgian marine environment was

treated by K. Wille (Merelbeke, Belgium). The results, derived from the INRAM project, indicated the presence of certain pharmaceuticals and perfluorinated substances in Belgian marine waters as well as in biota including mollusks, shrimp, and oysters.[28,29]

Court cases and economy

M. Nielen (Wageningen, the Netherlands) shared his experiences with recent court cases in the Netherlands. He made clear that it is not always easy to defend analytical residue data in court; attorneys and judges often read and interpret regulations such as the 2002/657/EC differently than chemists do.[30] In that aspect, he made some recommendations to include in the 2002/657/EC.

As the last speaker of the symposium, H. De Brabander (Merelbeke, Belgium) expanded on the economics of residue analysis. The speaker concluded that the nominal prices (the price on an invoice at a certain time) have remained relatively stable over a period of 40 years. By inflation and cost increase, the relative prices have decreased substantially and laboratories have reacted by increasing their productivity.[31]

Conclusions

During the symposium, the state of the art in residues analyses, anno 2010, was presented. From an analytical point of view, the use of UHPLC with (affordable) accurate mass (such as Orbitrap and ToF technology) is constantly increasing. These hyphenated techniques are more often being used in combination with biological detection systems and 'omic' techniques (amongst others, metabolomics). Moreover, these new analytical techniques, allowing continuously lower detection limits, lead to the observation that more and more xenobiotic substances turn out to be naturally occurring in some matrices and/or circumstances evidently at very low concentrations. Nortestosterone and boldenone have been known to be semi-endogenous for some time. However, thiouracil (in urine of farm animals and humans), chloramphenicol (in some herbs), and semicarbazide (in the shell of prawns) are newcomers. In addition, attention needs to be paid to depletion studies, legislation, court cases, and last but not least, the economic aspects of residue analysis.

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