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Popular Article

A Simple and Rapid HPLC Method for the Multi-Determination of Residue in Veterinary and Dairy Industries

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Abstract

Food safety issues have been attracting increasing public attention with the occurrence of large numbers of food safety incidents in recent. Veterinary drugs, pesticides and other chemical contaminants have become some of the most serious food safety problems. Different analytical methods have been applied to multi-residue detection of veterinary drugs and pesticides in animal-derived. To date, high performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) techniques has offered abundant qualitative and quantitative information from ‘‘target’’ to ‘‘non-target’’ analysis of these residues in foodstuffs. distinction between vaccination against paratuberculosis and tuberculosis.

Introduction

Until last two decades exposure of pharmaceuticals to environment has not been considered a big concern because they were thought to be safe. Nowadays, however, they are referred to as the emerging contaminants. The fact that these compounds are not in the regulatory list of environmental pollutants resulted in comparatively little attention paid to them. These drug residues may present great hazards to human health, and thus, veterinary drug and pesticide residue analysis is necessary for the protection of consumer health. Additionally, residue analysis also plays important roles in guaranteeing high-quality food products and international trade.

Rapid methods and automation for the detection and characterization of chemical and veterinary drug residues in foods of animal origin constitutes a dynamic area in food processing and is experiencing important developments mainly from the standpoint of food safety. Residues from these substances may be present in edible tissues, milk and eggs for human consumption and may exert different levels of toxicity on consumers when consuming them.

Antibiotics act as growth promoters but can contribute to an increased human exposure to antibiotics, development of pathogens with antibiotic-resistance and increased allergies due to its presence in foods. In fact, the presence of residual antibiotics in animal foods constitutes an important health risk because the increased microbial resistance detected in latest years.

Table 1. Lists of veterinary drugs and substances with anabolic effect, with some examples (Council Directive 96/23/EC)

Group A: substances having anabolic effect	Group B: veterinary drugs
Stilbenes (diethylstilbestrol) Antithyroid agents (thiouracils)	Antibacterial substances Sulfonamides and quinolones
Steroids; Androgens (trenbolone acetate), Gestagens (melengestrol acetate), Estrogens (17-b estradiol)	Other veterinary drugs; Antihelmintics, Anticoccidials, Carbamates and pyrethroids, Sedatives, Non-steroideal anti-inflammatory drugs, Other pharmacologically active substances (dexamethasone)
Resorcycilic acid lactones (zeranol) Beta-agonists (clenbuterol) Other compounds (nitrofurans)	

Chromatography is a common term used for a family of laboratory techniques, used for separation of the components of complex mixtures. Chromatography involves a sample being dissolved in a mobile phase, which may be a gas (in case of gas chromatography) or a liquid (in case of liquid chromatography). The mobile phase is then forced through an immobile stationary phase called the column. The mobile and stationary phases are chosen such that components of the sample have differing solubility in each phase. A component which is quite soluble in the stationary phase will take longer to travel through it than a component which is not very soluble in the stationary phase but very soluble in the mobile phase. As a result of these differences in mobility, sample components will become separated from each other as they travel through the stationary phase.

Different analytical methods have been applied to multi-residue detection of veterinary drugs and pesticides in animal-derived. To date, high performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) techniques has offered abundant qualitative and quantitative information from ‘‘target’’ to ‘‘non-target’’ analysis of these residues in foodstuffs.

The separation principle of HPLC is based on the distribution of the analyte (sample) between a mobile phase (eluent) and a stationary phase (packing material of the column). Depending on the chemical

structure of the analyte, the molecules are retarded while passing the stationary phase. The specific intermolecular interactions between the molecules of a sample and the packing material define their time "on-column". Hence, different constituents of a sample are eluted at different times. Thereby, the separation of the sample ingredients is achieved.

A detection unit (e.g. UV detector) recognizes the analytes after leaving the column. The signals are converted and recorded by a data management system (computer software) and then shown in a chromatogram. After passing the detector unit, the mobile phase can be subjected to additional detector units, a fraction collection unit or to the waste. In general, a HPLC system contains the following modules: a solvent reservoir, a pump, an injection valve, a column, a detector unit and a data processing unit. The solvent (eluent) is delivered by the pump at high pressure and constant speed through the system. To keep the drift and noise of the detector signal as low as possible, a constant and pulse less flow from the pump is crucial. The analyte (sample) is provided to the eluent by the injection valve.

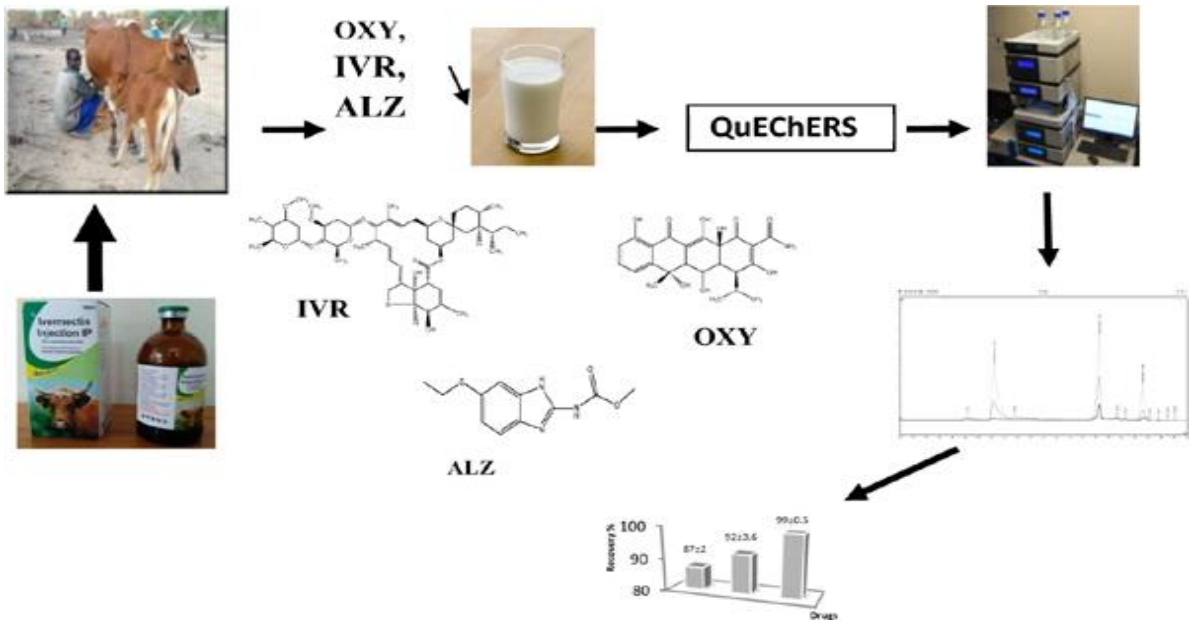

Figure 1: Method of Veterinary Drug Residue Analysis in Raw Milk by RP-HPLC-UV (Lekweiri *et al.*, 2020)

The highly toxic Aflatoxin M1 (AFM1) is most often detected in milk using an Enzyme-Linked-Immunosorbent Assay (ELISA) for screening purposes, while High-Performance Liquid Chromatography with Fluorescence Detector (HPLC-FL) is the reference method used for confirmation.

Table 2. Main advantages and disadvantages of HPLC

Advantages	Disadvantages
Short time (few min/sample) to obtain the results	Expertise required
Sensitive	Need of sample preparation (extraction and filtration, addition of internal standard, etc.)
Specificity depending on detector	High initial investment (equipment)
Automatisation leading to higher productivity	Cost of column
Possibility to find more information from spectra when using diode array detector	

Conclusion

The HPLC method is accurate, simple, rapid, and economic, and can be applied as a screening method in the determination of drug residues in veterinary and dairy products. So, it might be possible to be protected from residues, which are important for public health, and to reduce economic risks.

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