Liquid chromatography-tandem mass spectrometry for performing confirmatory analysis of veterinary drugs in animal-food products

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This review focuses on recent developments and trends in liquid chromatography coupled to mass spectrometry, with a particular emphasis on tandem mass spectrometry and the new criteria established by the European Union for performing confirmatory analysis of veterinary drugs in animal-food products. The combination of liquid chromatography and tandem mass spectrometry allows unequivocal identification of traces of antibiotics and antibacterial agents in complex biological matrices, such as honey, eggs, milk and meat. The sensitivity of the coupling is particularly useful for confirming the presence of banned substances that require limits of detection as low as possible.

Keywords: Animal food products; Antibiotic; High-pressure collision cell; Mass analyzer; Veterinary drug

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1. Introduction

In modern systems of livestock breeding, veterinary drugs are employed for therapeutic, metaphylactic, prophylactic and growth promotion purposes [1,2] and are administered as feed additives or via the drinking water. The occurrence of unwanted residues in edible products can be the result of illegal use, in the cases of prohibited medicines, or of failure to respect the proper withdrawal times before butchering, in the cases of permitted medicines. Adverse effects for consumer health are connected with the intrinsic toxicity of a drug and its metabolites; some chemotherapeutics are also suspected of being carcinogenic, but the main concern is the selection of resistant bacteria that, through the food chain, can be transferred to humans [3].

The control of abuse is at present based on screening procedures, using radio or enzyme immunoassays, which are often too specific to allow effective identification of multi-analytes; these methods provide only semi-quantitative analysis and sometimes give rise to false positives, but they continue to be used because of their simplicity in sample preparation, their sensitivity, their speed and their cheapness.

By contrast, chromatographic techniques allow quantitative multi-analyte determinations and compound identification is based on the different retention times. In spite of the high resolving power of gas chromatography (GC), provided by the capillary columns, liquid chromatography (LC) is the method of choice for determination of antibiotics, which are rather polar, non-volatile and sometimes heat sensitive but, when complete separation of all components of a mixture is not possible, the quantitative analysis lacks precision and accuracy. The power of a mass spectrometer as chromatographic detector lies in its capability to determine, by means of the molecular weight, the precursor ion (if stable enough) and its products of fragmentation, which also give structural information. The combination of LC with mass spectrometry (MS) allows, besides more definitive identification, the quantitative determination of compounds that, because they belong to the same class, could not be fully resolved chromatographically. In addition to the very high level of specificity, this combined technique is also sensitive

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and sometimes requires less than 1 picogram (pg) of material.

Based on these performances, the European Union (EU) relies on MS detection for unequivocal identification of chemical residues in foodstuff. European Commission (EC) Decision 2002/657/CE [4] states that ''methods based only on chromatographic analysis without the use of molecular spectrometric detection are not suitable for use as confirmatory methods''. At present, confirmation that a sample is positive, verified with bioassay techniques, is based on GC–MS; this technique, routinely used for the last 35 years, is often a time-consuming method of analysis and has left the way open to LC–MS, which does not require polar, non-volatile and thermally-unstable compounds to be derivatized.

Among the different types of interfaces developed in the last 30 years, particle beam (PB), thermospray (TS), electrospray (ES) and atmospheric pressure chemical ionization (APCI) have been the most effective in LC–MS combinations and have had the greatest commercial success. The technological problems in interfacing, the principles, the instrumentation and the applications of LC–MS have been extensively explained in books [5–7] and reviews [8–10]. In particular, the use of LC–MS in the analysis of antibiotic residues in edible products was reviewed by Niessen [11] and Kennedy [12] in 1998 and by Di Corcia [13] in 2002. From the analysis of how LC–MS works when aimed at detecting antimicrobial agents in foods, the result is that almost all of them use atmospheric pressure ionization (API) sources; the ES ionization (ESI) source is particularly suitable because of the polar nature of antibacterial drugs.

Below, in succession, we will describe some developments in API-interface technology and, in greater detail, advances in mass-analyzer technology that could have interesting consequences for confirming the presence of veterinary chemotherapeutics, mostly those banned by law.

2. Advances in interface technology

The success of the API interfaces is due solely to the well-known advantages that result when operating at atmospheric pressure. In recent years, there have been two trends in the development of ESI interfacing: high flow-rate sources, used for applications in food and environmental field; and, low flow-rate sources (micro- and nano-ESI), dedicated to protein analysis. In the former, application of a hot gas flow – in concurrent flow (a Z-spray interface) or perpendicular to spray (a turbo-ion spray interface) – enhances droplet desolvation and generally increases the S/N chromatographic ratio. In a multi-residue method developed by our group to detect sulfonamides (SAs) in raw meat and infant foods by means of a turbo-ion spray interface [14], we verified that the use of drying gas (temperature set up at 300°C) enabled the S/N ratio to increase by approximately five times.

ESI and APCI interfaces are the sources of choice to promote the ionization of antibiotics; they complement each other well with regards to polarity and molecular-mass of analytes, but have little effect with very low polarity substances which, not being prone to undergo acid-base reactions, are difficult to detect or are determined with poor limits of detection (LODs). Among several approaches that have been made to extend the applicability range of LC–MS with atmospheric pressure techniques, the most interesting is the atmospheric pressure photoionization (APPI) interface [15–17] that is the youngest soft ionization method in MS. APPI can be considered as a modified APCI source: a heated nebulizer, that operates at temperatures between 300°C and 450°C, vaporizes the LC effluent; the corona discharge is replaced by a gas-discharge lamp which, emitting photons ($h\nu$ = 10 eV) in the UV region of the electromagnetic spectrum, can selectively ionize most analytes in the presence of the common LC solvents. The addition in large quantities of a photoionizable substance, called a dopant, can greatly enhance the analyte signal; toluene, followed by acetone, are the preferred dopants. This new interface could be applied to the analysis of some classes of antimicrobials with an high electron affinity, such as amphenicols, nitroimidazoles and nitrofurans, that are provided with substituents with such characteristics. We found in the literature one APPI application for detecting chloramphenicol (CAP) in fish meats [18]; CAP was observed in negative ion mode as an $[M - H]^-$ ion that, as a consequence of in-source collision-induced dissociation (CID) fragmentation, generated the same ions produced by ESI and APCI sources. The authors observed, on comparing the APPI and APCI sources, that the former gave a better S/N ratio, a higher selectivity and a lower matrix effect, which eliminated the need for matrix-matched standards.

3. Advances in analyzer technology

Although the mild ionization conditions of API interfaces can usually guarantee the determination of molecular weight, the lack of fragmentation precludes useful structural information. A solution to this problem is the fragmentation activated in the ion transport region (up-front or in-source CID) or the use of tandem MS. Although in-source CID certainly is a useful technique, especially when used for pure compounds or with an efficient chromatographic separation, the method also has clear limitations. By means of in-source CID, all ions present are dissociated, while, in CID in the collision cell of an MS–MS instrument, the selection of a particular