Nowadays, ambient mass spectrometry techniques such as desorption electrospray ionization (DESI) and direct analysis in real time (DART) have been incorporated as alternative ionization sources in mass spectrometry (MS). These techniques are specifically designed for the direct analysis of compounds from sample surfaces by ionizing them outside the instrument and recording mass spectra. Ordinary samples are analyzed in their native environment without tedious sample treatments or chromatographic separations. With these techniques, the analysis can be performed in few seconds, which is a significant advantage when compared to more conventional analytical methods [1].

The ionization mechanism occurring in DESI has been described as “droplet pick-up” [2]. The ionization of analytes from the sample starts by the electrospray generation of charged droplets from a selected solvent. These charged droplets are further directed as a high-velocity gas jet into the sample surface, thus wetting this surface and dissolving the analytes. Ejected secondary charged micro-droplets will finally generate ions in the gas-phase that will be analyzed by mass spectrometry. DESI is a soft ionization source, hence very low in-source collision-induced dissociation (CID) occurs and no fragments appear in the spectra. Mainly, protonated molecules and alkali metal or ammonium adduct ions in positive ion mode, and deprotonated molecules when acquiring data in negative ion mode are generated. Thus, every ion (isotope cluster ion) in the mass spectrum could be assigned to one individual compound.

Veterinary drugs are widely used across developed countries to treat animals and protect their health. As an example, Figure 1 shows several veterinary drugs frequently used in medicated feeds. Despite the regulations set for feed business operators (Regulation EC No 183/2005), it is generally acknowledged that during the production of mixed feeds a certain percentage of the feed batch remains in the production circuit and these residual amounts can contaminate the subsequent feed batches. This cross-contamination leads to unintentionally drug exposure of non-treated animal species. The analysis of cross-contaminated feeds requires a laborious sample treatment step previous to sensitive and selective analytical methods for the accurate identification and confirmation of the veterinary drugs in such complex matrices. Hence, the development of multiclass and fast screening methods is needed in order to increase the productivity in agricultural and food laboratories.

Today, liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) using triple quadrupole and ion trap analyzers is the technique most currently used for the determination of veterinary drugs in both food and feed [3]. This technique provides in addition to confirmation capabilities high sensitivity and selectivity for the analysis of target compounds. However, most of the target LC-MS/MS methods require the optimization of acquisition parameters for each compound, resulting in extensive method development (time-consuming) when multiple compounds in a single sample must be analysed. Furthermore, the number of compounds to be monitored in a single run is limited and only those analytes included in the target MS-acquisition method are detected, making impossible the retrospective data analysis. In addition, matrix effects caused by coeluting interfering matrix components, which can produce a reduction (ion suppression) or even an increase (matrix enhancement) of the analyte response, are a major problem when analyzing these complex samples. To overcome some of these limitations, reducing the effect of matrix interferences and maximizing information from the sample, high resolution mass spectrometry (HRMS) can be used. However, only few methods can be found in the literature regarding HRMS applied to the analysis of feed samples [4]. An additional advantage of HRMS is that its high selectivity could make possible the use of simple generic sample preparation procedures even without chromatographic separation.

![Figure 1: Chemical structures of some veterinary drugs frequently used in medicated feeds.](image-url)
The aim of this work is to study the applicability of DESI coupled to HRMS (Orbitrap) for the screening of veterinary drugs in cross contaminated feed samples.

Two main groups of DESI-MS conditions must be optimized prior to analysis: geometrical parameters and conditions related to the electrospray source. The first group includes nebulization capillary angle, the tip distance to the sample surface and the distance to the mass spectrometer inlet. The second group refers to the nebulizing gas pressure, the electrospray solvent composition and the electrospray solvent flow rate. In this work, the optimization of the multiple DESI parameters has been carried out by an experimental design to simplify the optimization procedure since most of these parameters are interdependent. Based on our experience, the use of well-known and easily ionisable standards such as rhodamine or bradykinin is not enough to optimize DESI ion source conditions and to transfer them to the analysis of complex matrices. For instance, the wetting of the sample surface depends on the surface sample nature and porosity, the electrospray solvent flow rate and the nebulizing gas. Thus, in this work DESI ion source conditions were always optimized using blank feed samples spiked with a set of veterinary drugs. As an example, Figure 2 shows the optimum DESI ion source conditions used for the analysis of 2 µL feed sample extract placed in a glass slide (7.1 mm² surface area HTC polymeric printed spots).

Figure 2: Optimum DESI source conditions for the analysis of veterinary drugs in a feed sample.

The feed samples have a dusty texture, hence different sample manipulation strategies were evaluated for the screening of the veterinary drugs. One of the strategies was to prepare pressed feed pellets with the powdered feed to obtain a smooth surface to be screened with DESI-HRMS. However, the dusty texture of the pressed pellets did not produce good results since the nebulizing gas damaged the surface and contaminated the mass spectrometer transfer line. The optimum strategy was a simple and fast solid-liquid extraction (acetonitrile) and the direct analysis of an aliquot of the extract after being dried on the HTC polymeric printed spot. To evaluate the applicability of the proposed DESI-HRMS method, more than 40 feed samples collected from farms and feed mills in Catalonia (Spain) were analyzed. DESI-HRMS allowed the detection of veterinary drug cross-contamination in several medicated and not medicated feed samples at µg/g concentration levels. The results obtained by the proposed DESI-HRMS method were validated using a well-established UHPLC-MS/MS method [5]. Figure 3 shows the mass spectrum of a narasin medicated feed (45 µg g⁻¹) cross-contaminated with monensin (3.5 µg g⁻¹). The results obtained in this study demonstrate that the proposed DESI-HRMS method is fast and suitable for the screening of veterinary drug cross-contamination in feed samples by using a simple sample extraction procedure. This method will improve high throughput analysis in quality control laboratories.

Figure 3: Mass spectrum of a narasin (NAR) medicated feed cross-contaminated with monensin (MON).

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