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Multi-residue quantification of veterinary drugs in milk with a novel extraction and cleanup technique: Salting out supported liquid extraction (SOSLE)

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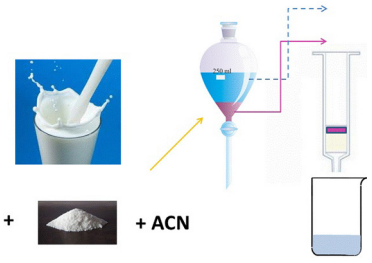
HIGHLIGHTS

- We are describing a novel liquid/liquid extraction technique.
- The technique can extract compounds of widely different polarities.
- Recovery and clean-up is equal or better than provided by other methods.
- A multi-residue veterinary drug method for milk was validated.

GRAPHICAL ABSTRACT



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ABSTRACT

A quantitative liquid chromatography coupled with high-resolution mass spectrometry method was developed for the determination of more than one hundred compounds belonging to a variety of veterinary drug classes in bovine milk. Salting out supported liquid extraction (SOSLE), a novel extraction and cleanup technique, was introduced to ensure high extraction efficiency and good sample cleanup. The high salt (ammonium sulfate) concentration in the aqueous donor phase permits supported liquid/liquid extraction (SLE) with a relative polar organic acceptor phase (acetonitrile). This is different from traditional SLE, in which the need for phase separation results in the selection of organic solvents with intermediate polarities (e.g., ethyl acetate or dichloromethane). Hence, SOSLE is more efficient in recovering polar analytes than conventional SLE. SOSLE was also compared to classical approaches like solid phase extraction, QuEChERS and ultra-filtration. The proposed technique resulted in extracts of equal or superior cleanliness and with higher average recoveries than those obtained with QuEChERS or SPE. The recovery (median for all compounds) was 73% for QuEChERS, 83% for SPE and 91% for SOSLE. The most significant improvements were observed for polar analytes (penicillines, quinolones and tetracyclines) which are hardly recovered by QuEChERS. The chromatographic separation and detection was based on an ultra-high-performance liquid chromatography Q-Orbitrap system (Q-Exactive plus). The developed analytical method has been validated (based on the commission decision 2002/957/EC) as required for quantitative veterinary drug methods.

1. Introduction

Veterinary drugs are widely used in animal husbandry to ensure the health and the growth of animals. Residues of such drugs are not desirable, because of the threat that this will promote the proliferation of multi-antibiotic resistant bacteria strains [1]. Furthermore,

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antibiotics present in milk can have negative implication on microbial processes (e.g., cheese production). Therefore, milk should be free of antibiotics present at relevant concentrations. This is being controlled by analytical methods capable in monitoring an increasingly long list of possible veterinary drug residues.

Multiresidue veterinary drug methods developed for milk are currently based on a number of different extraction and cleanup principles. The most straightforward approach is the dilution of the milk sample with a solvent like acetonitrile, followed by the centrifugation and evaporation of the obtained supernatant organic extract [2–4]. Final extracts obtained by such a technique contain high concentrations of co-extracted endogenous matrix compounds, which can cause serious signal suppression effects in the interface between a liquid chromatograph and a mass spectrometer (LC–MS). The additional use of an ultrafiltration device has been proposed to remove high molecular compounds from the final extracts [5,6]. The available data permit the conclusion that ultrafiltration produces high recoveries for small analyte molecules, but poor recoveries for large analyte molecules [5,7]. Superior recoveries and acceptable signal suppressions have been obtained through a reversed-phase, solid-phase extraction (SPE) cleanup step [7–9]. A drawback of such reversed-phase-based SPE methods is the partial loss (break-through) of very polar compounds [7]. Matrix solid-phase dispersion (MSPD) [10,11] hase been reported as well. Good recoveries for polar β-lactams and quinolones were reported by MSPD [10,11]; however, no data were given for other veterinary drugs. Furthermore, no information regarding signal suppression is available, since these two reported methods utilize diode array technologies as detecting devices. The methodology of quick easy cheap effective ruged safe (QuEChERs), which was originally developed for pesticides, has recently been proposed for the analysis of veterinary drugs in a variety of different matrices [12–19]. However, QuEChERs seems to be less suited for recovering polar veterinary drugs like penicillines, tetracyclines and quinolones [14,18,19]. An extensive removal of interfering milk proteins by the highly selective technique of online turboflow has also been reported [20]. There is currently only limited data available regarding the polarity ranges of compounds, which can be quantitatively analyzed by turboflow [20]. Hence, the suitability of turboflow for extensive multi-residue methods has not yet been proven.

This paper proposes an alternative cleanup strategy based on the concept of supported liquid/liquid extraction (SLE). Unlike conventional liquid/liquid extraction, SLE uses a solid support (chemically inert diatomaceous earth) to retain the aqueous donor phase. The obtained high-phase surface area permits easy extraction of analytes from the aqueous phase (donor phase) into an added, immiscible organic extraction solvent (acceptor phase). SLE is a cleanup technique that has been used for the analysis of pesticides [21–23] and drugs of abuse [24] and for the general bioanalysis of pharmaceutical compounds [25]. Although the technique has received some positive feedback (such as “Supported liquid extraction: The best-kept secret in sample preparation” [26]), it has clearly been less popular than SPE. As a matter of fact, SLE was not even mentioned in some recent review papers focusing on the sample preparation of veterinary drug residues [27,28]. When compared to QuEChERS, SLE results in significantly poorer recoveries for some analytes [29]. The reason for this is the requirement in SLE to utilize an aqueous donor phase and an immiscible organic acceptor phase. The aqueous solution is a relatively poor solvent with respect to quantitatively extracting apolar analytes from a sample matrix. On the other hand, the required immiscible acceptor phase may be too apolar to re-extract some of the polar analytes out of the donor phase.

This problem has been addressed by the proposed concept of salting out supported liquid extraction (SOSLE). To our best knowledge, the concept of “salting out” [31] was never combined with the technique of SLE. In SOSLE, a mixture of acetonitrile and water is used to ensure the near quantitative extraction of analytes from the sample. Such a solvent is capable of extracting a wide polarity range of analytes from the matrix. After centrifugation, solid ammonium sulfate is added to induce a phase separation of the solvent mixture. The resulting heavier aqueous phase is added to an SLE cartridge. After equilibration, the compounds retained on the column are eluted with the supernatant organic phase. Final extraction and elution of the analytes is achieved through additional volumes of acetonitrile. The combined eluates are evaporated to produce the injection-ready extract.

The proposed method has been compared to other published methods dealing with the determination of veterinary drugs in milk samples. The focus was the recovery of a wide polarity range of analytes, since some polar veterinary drugs (e.g., penicillin’s and tetracycline’s) are of particular importance in milk. Furthermore, attention has been paid to the selectivity of the cleanup, as indicated by the total ion current (TIC) of the chromatographically separated extracts and the extent of analyte-specific signal suppression effects. The developed LC-Q-Orbitrap analytical method has been validated, based on the concept of the commission decision 2002/657/EC. It is the aim of the paper to present an analytical methodology, providing high recoveries for compounds of various polarities and at the same time ensuring good clean-up, high speed and low cost of consumables.

2. Materials and method

2.1. Standards and stock solutions

The veterinary drug reference substances are listed in Table 1. They were obtained from various sources [30] and were of the highest available purity. These compounds belong to the families of benzimidzoles (bz), quinolones (ch), macrolides (ml), nitroimidazoles (ni), penicillines and cephalosporines (pc), sulfonamides (sa), tetracyclines (tc) and compounds not assigned to a particular drug family (vs).

Individual stock solutions (1000 mg L^{-1}): These were prepared by accurately weighing 50 mg of reference substance into a 50 mL volumetric flask. Depending on their specific solubility properties, the compounds were dissolved and diluted to volume with acetonitrile, dimethlysulfoxide or methanol.

Mixed spike solution (1000/10,000 $\mu g\,L^{-1}$): The solution was prepared by transferring 0.1 mL of the individual stock solutions (only those compounds listed in Table 1 under “conc. group = A”) into a 100 mL volumetric flask. Afterwards, 1.0 mL of the individual stock solutions (only those compounds listed in Table 1 under “conc. group = B”) were transferred to the same flask and diluted to volume with dilution solution for standards. The reason for these two concentrations is the fact that the compounds have very different MRL levels (see Table 2).

Mixed spike solution (100/1000 $\mu g\,L^{-1}$): 2.5 mL of the mixed spike solution (1000/10,000 $\mu g\,L^{-1}$) was transferred into a 25 mL volumetric flask and diluted to volume with the dilution solution for standards.

Reference solution (50/500 $\mu g\,L^{-1}$), respectively (25/250 $\mu g\,L^{-1}$), respectively (5/50 $\mu g\,L^{-1}$), respectively (1/10 $\mu g\,L^{-1}$), respectively (0.2/2 $\mu g\,L^{-1}$): Appropriate volumes of the mixed spike solution (100/1000 $\mu g\,L^{-1}$) was diluted with the dilution solution for standards to reach the desired concentration.

2.2. Reagents and solvents

The acetonitrile, formic acid (98%), ammonium hydroxide (25%), methanol, dimethylsulfoxide (DMSO), oxalic acid,