Reviews describing the state of the art and future trends in antibiotic residue analysis point out a shift towards the use of multi-class methods with modern liquid chromatography coupled to mass spectrometry instruments such as UPLC/ToF MS, that allow the analysis of more than 100 different drug residues. These analytical methods therefore apply less specific sample preparation steps including ‘dilute-and-shoot’ or protein precipitation combined with ultrafiltration, that comply with the QuEChERS methodology (quick, easy, cheap, effective, rugged and safe). These techniques provide significant benefits, but several problems still do exist. A first pitfall is the possibility of ion suppression and matrix effects which may be responsible for quantification errors. Assessment of the impact of these effects is not included in international guidelines for validation of analytical methods (e.g. VICH and 2002/657/EC). A second pitfall concerns the simultaneous extraction of antibiotics with widely different polarities. A third pitfall is that there are often compromises needed in LODs, chromatography and quantitation due to poor linearity.

The author’s analytical lab can testify to the benefits, but also to possible drawbacks of these trends in the residue analysis of tetracyclines in particular, which are frequently used in veterinary medicine. These antibiotics can readily chelate to metal ions and can interact with silanol groups on traditional silica-based LC columns. Another problem is that chlortetracycline and doxycycline peaks frequently show excessive fronting. In addition, epimerization and keto-enol tautomerism result in the formation of keto- and enol-forms of chlortetracycline, doxycycline and their 4-epimers. This phenomenon is rarely mentioned in the literature, and complicates the quantification of chlortetracycline and doxycycline. The implementation of a new technique, i.e. UPLC-MS/MS has resulted in a significant increase in chromatographic resolution between keto- and enol-forms. However, since standards of these keto-enol forms are not available, it is a priori not possible to quantify both tetracyclines content in incurred plasma and tissue samples, considering a different keto-enol ratio in incurred samples compared to spiked samples or standards used for quantification. The possible impact of this phenomenon on quantification and residue monitoring will also be discussed.