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Acquisition and data-processing parameters in suspect screening of antibiotics using LC-HRMS

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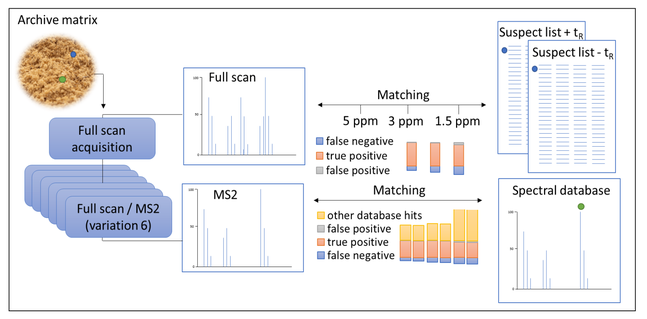
Introduction

For years, targeted analysis by LC-MS/MS has been used to monitor specific veterinary drugs in products of animal origin. Now, monitoring strategies start shifting towards a non-targeted, more risk-based approach, as is mandatory according to the official controls regulation (EU) 2017/625 [1]. This shift has pushed the utilization of high-resolution mass spectrometry (HRMS) and suspect screening, an approach for processing untargeted data in which reference standards are not a necessity.[2,3] Studies that investigate the performance of suspect screening workflow strategies for the detection of veterinary drugs in complex matrices are however still scarce. In this work, a systematic assessment was carried out in feather meal matrix, using both an in-house suspect list and the online mzCloud™ database.



Systematic assessment results

- Data processing using variations in mass tolerance in the suspect list pointed out that lower mass tolerance, especially 1.5 ppm, results in more false positives.
- The application of a retention time in the suspect list (mass tolerance, 5 ppm) leads to more correct identification and therefore aids in lowering the false positive rate.
- The acquisition method resulting in the least false positives and most true positives is method 6 in figure 1.
- The use of the mzCloud™ database (figure 1, bottom row) did not result in a large increase of false positives compared the suspect list including retention times (figure 1, top row).

↳ Addition of MS2 data matching compensates for the lack of a retention times in a suspect list.

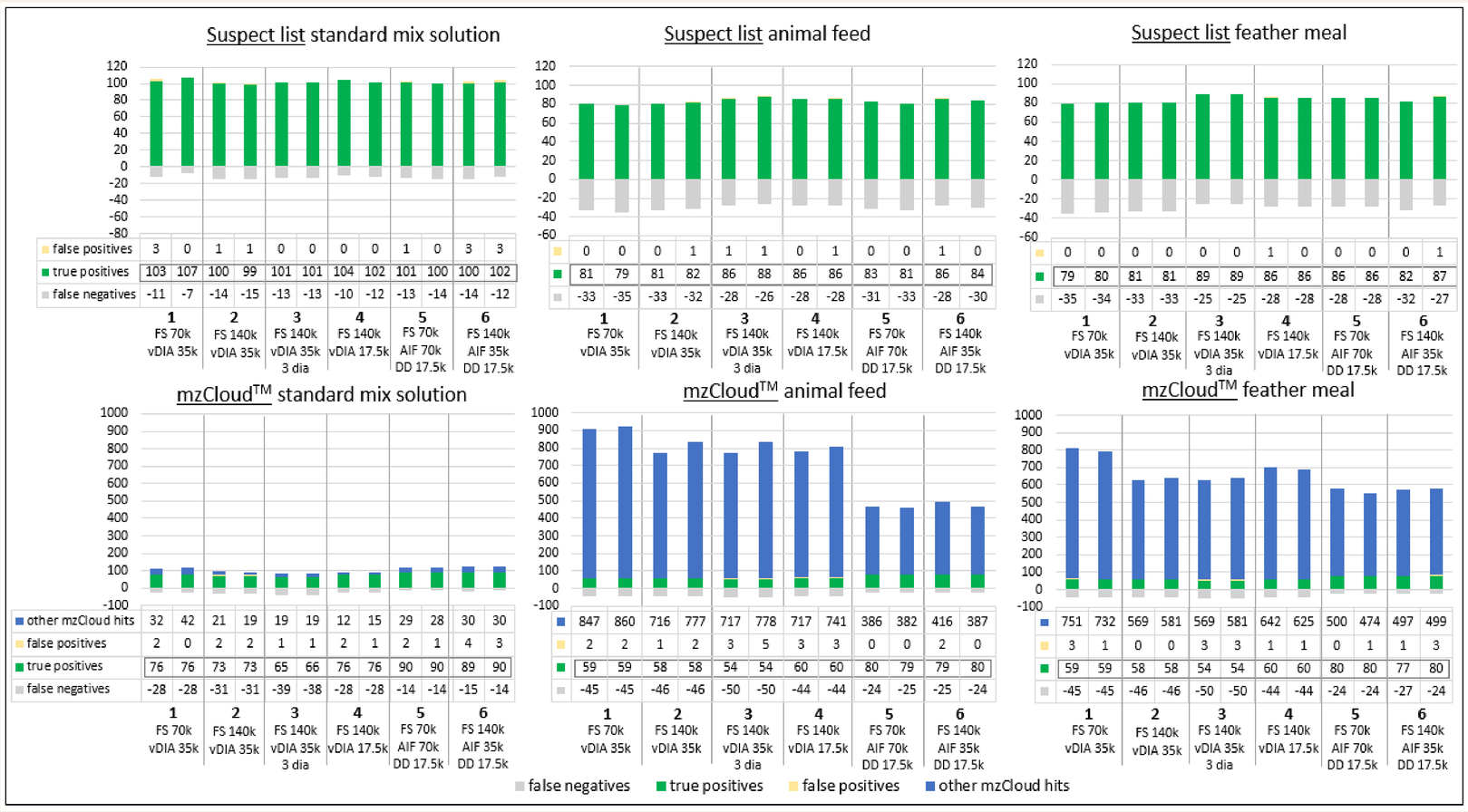


Figure 1. The number of true positives, false negatives and false positives in standard mix solution, fortified animal feed and feather meal matrix based on 6 different acquisition methods using either a suspect list workflow (including retention times (±0.1 min), 5 ppm mass tolerance) or the mzCloud™ database. Using the latter, the number of other mzCloud™ hits were also evaluated.

Pilot study results

Using method 6 (figure 1), fourteen different antibiotics were detected and confirmed in feather meal samples used in a pilot study (table 1). Three antibiotics were found based on only an mzCloud™ hit; azithromycin (macrolide) and gatifloxacin and levofloxacin (fluoroquinolones). These compounds were not included in the suspect list nor in the scope of the routine LC-MS/MS monitoring method (x^b in tabel 1). Standards were bought and the identity of all three compounds could be confirmed according to (EU) 2002/657/EC [4].

Table 1. Results of confirmed antibiotics in imported feather meal samples. Suspect screening results are a match with the suspect list (a) or the online mzCloud™ database (b). Concentrations (µg kg^{-1}) are only shown if compounds are in scope of the LC-MS/MS method. Other confirmed compounds are indicated 'x'.

Sampe	1	2	3	4	5	6	7
Azithromycin						x^b	
Ciprofloxacin	29^a		7^a		18^a		$500^{a,b}$
Doxycycline		89		$>500^{a,b}$			
Enrofloxacin	$>1000^{a,b}$		$200^{a,b}$		$380^{a,b}$		
Gatifloxacin						X^b	
Levofloxacin						X^b	
Sulfadiazine						5	
Sulfadimethoxine	9				30		
Sulfadimidine						$61^{a,b}$	
Sulfamethoxazole						8^a	
Sulfaquinoxaline						$150^{a,b}$	
Tiamulin	3		$180^{a,b}$			$>500^{a,b}$	
Tilmicosin		$>500^{a,b}$	41				
Trimethoprim	$30^{a,b}$		3		$30^{a,b}$	$250^{a,b}$	

Conclusion

The developed suspect screening method was found to be fit for the purpose of finding unexpected antibiotics in feather meal and the developed strategy could greatly advance the early detection of the application of unexpected veterinary drugs.

References

[1] European Commission. (EU) 2017/625. Off. J. Eur. Union. 2017;L95: 1–142.

[2] Caballero-Casero *et al*,. Trends in Analyti Chem. 2021;136:116201.

[3] Pourchet *et al.,* Environ. Int. 2020;139:105545.

[4] European Commission. (EU) 2002/657/EC. Off. J. Eur. Union, 2002;L221: 8–36.

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