A SIMPLIFIED MULTI-CLASS METHOD FOR SIMULTANEOUS DETERMINATION OF GROWTH PROMOTERS BY LC-MS/MS

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Introduction

Veterinary drugs are widely used in livestock to treat disease, maintain animal health, promote growth, and improve meat quality and yield. The use of these drugs may leave residues in the food. Foods containing the residues may cause adverse health effects in humans. Therefore, government regulatory authorities control the use of veterinary drugs by approving or registering safe uses and monitoring food for unsafe or prohibited residues. In 1981, with Directive 81/602/EEC, the EU prohibited the use of substances having a hormonal action for growth promotion in farm animals. Also, the directives come into force in the following years, 96/22/EC and 2003/74/EC, confirm the prohibition of those substances.

Multiclass-multiresidue methods for veterinary drugs are scarce due to a number of analytical challenges including the lack of volatility and chemical stability of most drugs, wide polarity range among the various drugs and some drugs have to be determined at rather low concentration. Because of these difficulties most of the veterinary drug residue methods published focuses on one class of compounds such as b-agonists, quinolones and stilbens (Lohne 2013). Regarding simultaneous determination of different classes of growth promoters, there are only a limited number of methods published (Yang, Y. 2009). Most of these methods including multi step solid phase extractions are developed for determination of single class substances and their metabolites. There is a great demand for simple, rapid and multi-class multi residue methods. In this present study, a multi-class multi-residue LC-MS/MS method was developed for the determination of growth promoters. The developed method was validated according to the requirements of 2002/657/EC.

Column	Phenomenex Luna C18 100x2,1mm 1,9 µm			
Enjection volume	20 mcL			
Gradient program	time	% A Mobile Phase (su)	%B Mobile Phase (metanol)	Flow Rate (mL/min)
	0	50	50	0,5
	2	50	50	
	6	0	65	
	8	0	100	
	8.1	0	100	
	15	50	50	
Oven Temp.	30 °C			

Validation Results

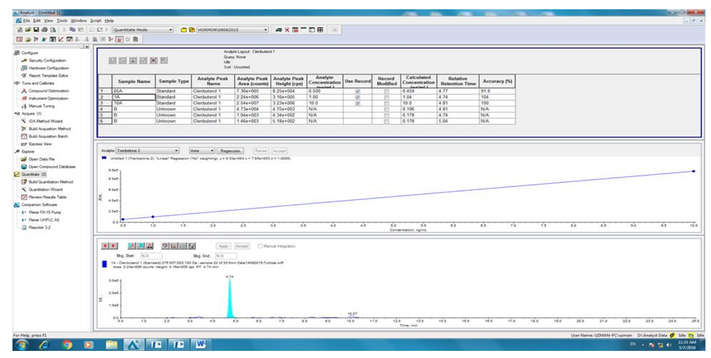
analyte	precursor ion, (m/z)	fragment ions, (m/z)	intraday precision (RSD%, n=6)	interday precision (RSD%, n=18)	average recovery (%) (n=18)	CCα (ppb)	CCβ (ppb)
17-alfa ethnylestradiol	279,3	133, 158.6	12	16	85	1,1	1,2
17- alfa-estradiol	255	159, 133	10	15	98	1,1	1,0
17-beta estradiol	255	159,133	10	15	98	1,1	1,1
diethylstilbesterol	269,5	135,107	16	15	88	1,1	1,2
hexestrol	(-) 269.1	133,5, 119	10	17	99	1,0	1,1
progesterone	316	109,97	16	11	101	1,1	1,2
17-beta testesterone	290	97,109	9	9	106	1,1	1,2
trenbelone	321,5	303	12	10	110	1,1	1,2
alfa zearalanol	323,6	305,287	12	15	86	1,1	1,2
alfa zearalenol	(-) 319.3	159,13	11	16	91	1,1	1,0
beta zearalanol	323,6	305,287	10	13	95	1,0	1,0
beta zearalenol	(-) 319.3	159,13	11	14	105	1,1	1,0
zearalenone	321	303,189	16	18	86	1,07	1,00

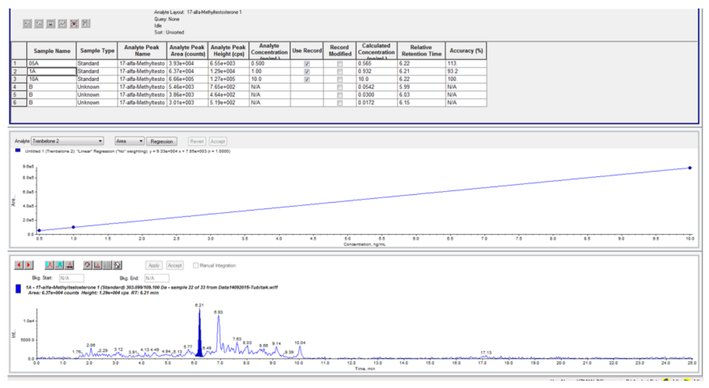
Results and Discussion

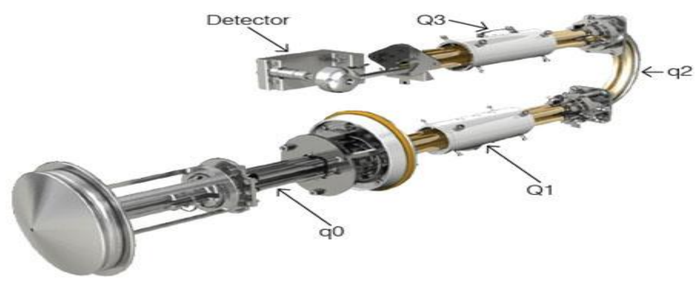
The selected analytes belonging to three different groups were extracted from bovine tissues using acetonitrile following enzymatic treatment with beta-glucuronidase (helix pomatia) over 15 hours. The results of the validation studies (intraday, interday, recovery, CCα and CCβ) obtained were within the acceptable limits. The validated method was successfully applied to the analysis of more than 200 real samples. Of these 22 were detected positive with 17-Beta testesteron.

Conclusions

A simple and reliable multiclass multiresidue LC-MS/MS method was developed for determination of compounds belonging to three different classes including steroids, stilbenes and zearenol and metabolites. The developed method was validated according to 2002/657/EC for simultaneous determination of 13 selected growth promoters in bovine tissue.







References

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