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A versatile liquid chromatography–tandem mass spectrometry system for the analysis of different groups of veterinary drugs

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Abstract

This work describes an analytical strategy, based on liquid chromatography–tandem mass spectrometry (LC–MS–MS), with a generic chromatographic system in which it is possible to analyse corticosteroids (CORT), β-agonists (BAG), chloramphenicol (CAP) and penicillins (PEN). The same mobile phase solvents and column were used, and only gradient tables and mass spectrometry acquisition methods were changed depending on the family of compounds to analyse. Different batches of final extracts, proceeding from different analytical methods, may be included in a single sequence and run overnight. Sequence programming and LC–MS–MS conditions are included and typical chromatograms are presented.

The proposed approach makes the performance of the analysis of veterinary drug residues more simple, cost-effective and less time-consuming.

Keywords: LC–MS–MS; β-Agonists; Corticosteroids; Penicillins; Chloramphenicol

1. Introduction

Liquid chromatography–tandem mass spectrometry (LC–MS–MS) is a powerful analytical tool, due to its high universality, specificity and sensitivity. The applicability of this technique in veterinary drug residue analysis has been proven during the last years by means of many scientific articles based on LC–MS–MS [1–6].

Routine laboratories for the control of veterinary drug residues in food producing animals have to analyse a large number of samples frequently handling different families of compounds. Some years ago, this situation required the use of different detection techniques, depending on the compounds to be analysed: liquid chromatography–diode array detection (HPLC–DAD) for penicillins (PEN) [7], chloramphenicol (CAP) [8] and aromatic amine β-agonists (BAG) [9], gas chromatography–mass spectrometry (GC–MS) for β-agonists [10] and corticosteroids (CORT) [11,12] etc. The analytical methods based on these techniques presented some disadvantages. For example, the lack of specificity and sensitivity when HPLC–DAD is used for banned compound determinations according to current EU legislation. Although GC–MS is a very specific and sensitive technique, derivatization is required to be applied to veterinary drug analysis which means time-consuming methods and sometimes lack of ruggedness. LC–MS–MS may offer a solution to all these problems, as it provides the possibility to analyse almost every compound and more over, due to its specificity, with very simple clean-up procedures.

That is the reason why overloading of the available LC–MS–MS capacity is nowadays a common situation in routine laboratories. On the one hand, the development of new methods and, on the other hand, the adaptation, for one reason or another, of all the existing methods based on techniques such as DAD, fluorescence and even GC–MS to LC–MS–MS are major objectives.

The aim of this work was the development of the most universal chromatographic system as possible, concerning

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Table 1
Gradient tables used for the chromatographic separation of the different veterinary drugs

Time (min)	A%	B%	C%	D%	Curve
Corticosteroids					
0	70	30	0	0	1
6	70	30	0	0	1
13	50	50	0	0	7
21	5	95	0	0	1
30	70	30	0	0	1
β-Agonists					
0	99	1	0	0	1
15	55	45	0	0	8
20	10	90	0	0	1
35	99	1	0	0	1
Chloramphenicol					
0	0	0	100	0	1
6	0	0	100	0	1
13	0	0	20	80	1
20	0	0	100	0	4
Penicillins					
0	99	1	0	0	1
12	30	70	0	0	5
13	30	70	0	0	1
18	5	95	0	0	1
25	99	1	0	0	1

Each method consisted of an analytical step to separate the compounds, a washing step (increasing the organic phase percentage) to clean the column, and a conditioning step to prepare the column for the next injection.

Table 2
MS–MS methods for the analysis of β-agonists (15), corticosteroids (7), chloramphenicol and penicillins (6)

Channel	Dwell	CV	Coll	Compound
β-agonists				
Function 1: ESI^+ (0–4 min); MRM of eight channels				
202 > 160	0.2	30	12	Cimaterol
220 > 202	0.2	20	10	Cimaterol
226 > 152	0.2	20	12	Terbutaline
226 > 170	0.2	20	11	Terbutaline
240 > 148	0.2	20	12	Salbutamol
240 > 166	0.2	20	16	Salbutamol
244 > 202	0.2	30	17	Zilpaterol
262 > 244	0.2	20	12	Zilpaterol
Function 2: ESI^+ (4–10.5 min); MRM of six channels				
234 > 160	0.2	20	16	Cimbuterol
234 > 216	0.2	20	10	Cimbuterol
304 > 135	0.2	20	18	Fenoterol
304 > 286	0.2	20	15	Fenoterol
301 > 203	0.2	35	17	Clenciclohexerol
319 > 301	0.2	20	12	Clenciclohexerol
Function 3: ESI^+ (10.5–11.5 min); MRM of four channels				
263 > 203	0.2	20	17	Clenproperol
263 > 245	0.2	20	12	Clenproperol
293 > 203	0.2	20	17	Hidroxymethylclenb
293 > 275	0.2	20	12	Hidroxymethylclenb
Function 4: ESI^+ (11.5–13.5 min); MRM of six channels				
277 > 203	0.2	20	15	Clenbuterol
277 > 259	0.2	20	10	Clenbuterol
286 > 268	0.2	20	10	D9-clenbuterol (IS)
302 > 164	0.2	20	15	Ractopamine
302 > 284	0.2	20	12	Ractopamine
307 > 289	0.2	20	12	D5-ractopamine (IS)
Function 5: ESI^+ (13.5–15.8 min); MRM of eight channels				
302 > 150	0.2	20	20	Isoxuprine
302 > 284	0.2	20	12	Isoxuprine
311 > 237	0.2	20	15	Mabuterol
311 > 293	0.2	20	12	Mabuterol
367 > 293	0.2	20	17	Brombuterol
367 > 349	0.2	20	12	Brombuterol
325 > 237	0.2	20	15	Mapenterol
325 > 307	0.2	20	12	Mapenterol
Corticosteroids				
Function 1: ESI^- (0–6.5 min); MRM of six channels				
437 > 347	0.3	20	15	Fluoroprednisolone (IS)
419 > 329	0.3	20	15	Prednisolone
329 > 295	0.3	40	20	Prednisolone
393 > 345	0.3	20	15	Triamcinolone
393 > 363	0.3	20	10	Triamcinolone
Function 2: ESI^- (6.5–13 min); MRM of eight channels				
433 > 343	0.3	20	15	Methylprednisolone
343 > 309	0.3	40	20	Methylprednisolone
451 > 361	0.3	20	20	Beta-dexamethasone
361 > 307	0.3	40	20	Beta-dexamethasone
469 > 379	0.3	20	15	Flumethasone
379 > 305	0.3	40	20	Flumethasone
467 > 377	0.3	20	15	Beclomethasone
467 > 341	0.3	20	20	Beclomethasone
Chloramphenicol				
Function 1: ESI^- (0–6 min); MRM of three channels				
321 > 152	0.3	30	15	Chlorampenicol
321 > 257	0.3	30	10	Chlorampenicol
326 > 157	0.3	30	15	D5-chloramphenicol (IS)
Penicillins				
Function 1: ESI^+ (0–6 min); MRM of four channels				
350 > 106	0.2	15	18	Ampicillin
350 > 192	0.2	15	15	Ampicillin
366 > 114	0.2	15	20	Amoxicillin
366 > 349	0.2	15	9	Amoxicillin
Function 2: ESI^+ (8–14 min); MRM of seven channels				
335 > 160	0.2	15	15	Penicillin G
335 > 176	0.2	15	10	Penicillin G
351 > 160	0.2	15	15	Penicillin V (IS)
402 > 160	0.2	15	15	Oxacillin
402 > 243	0.2	15	12	Oxacillin
436 > 160	0.2	15	15	Cloxacillin
436 > 277	0.2	15	12	Cloxacillin
470 > 160	0.2	15	15	Dicloxacillin
470 > 311	0.2	15	13	Dicloxacillin

Two MRM channels were monitorized for each compound, and one for internal standards (IS). Dwell (dwell time in seconds), coll (collision energy), CV (cone voltage) are included.