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Applikationsbericht

Meeting Challenging Requirements for the Quantitation of Regulated Growth Promoters Dexamethasone and Betamethasone in Liver and Milk

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Abstract

This application note describes the use of Waters Xevo TQ MS for the high sensitivity determinations of dexamethasone, at the European MRL level in food. In addition, the use of PICS acquisitions for further presence confirmation is explored.

Benefits

- Highly selective and sensitive analysis for dexamethasone and betamethasone in complex matrices.
- European MRLs and confirmation requirements (2002/657/EC) can be comfortably achieved using Xevo TQ MS.
- The high chromatographic resolution of the ACQUITY UPLC System facilitates the critical separation of the epimers dexamethasone and betamethasone.
- Additional spectral information can be obtained by data directed Product ion confirmation scan (PICS), which could help confirm the presence of banned substances.

Introduction

Ensuring consumer safety is a priority for governments, international regulatory bodies and organizations that process and handle products prior to consumption. Food safety issues arising from commodity products often become globally reported and have the potential to impact consumer confidence and trade at international levels.

Dexamethasone and betamethasone are synthetic glucocorticoids widely used in animal husbandry[1]. These epimeric compounds are licensed for therapy in veterinary practice, while their use as growth promoters is banned within the European Union (corticosteroids are listed in Annex I of European Council 96/23 - group B2f)[2].

In order to protect consumer safety, Maximum Residue Limits (MRLs) have been fixed for both molecules by the European Community in several matrices, for instance: 0.3 µg/L (ppb) in bovine milk and 2.0 µg/kg (ppb) in liver from different species[3].

The major challenge in the analysis of dexamethasone and betamethasone consists of performing an

efficient separation of both epimers and detecting and identifying these molecules at the required maximum residue limit (MRL). Malone *et al.*[4] and Li *et al.*[5] have illustrated efficient methods for the separation of both epimers.

This application note describes the use of Waters Xevo TQ MS for the high sensitivity determinations of dexamethasone, at the European MRL level in food. In addition, the use of PICS acquisitions for further presence confirmation is explored.

Experimental

Sample preparation

Sample extraction and purification procedure in liver is described in detail elsewhere[6]. Initial sample preparation for milk included a protein precipitation step which then followed the same procedure as liver (Figure 1).

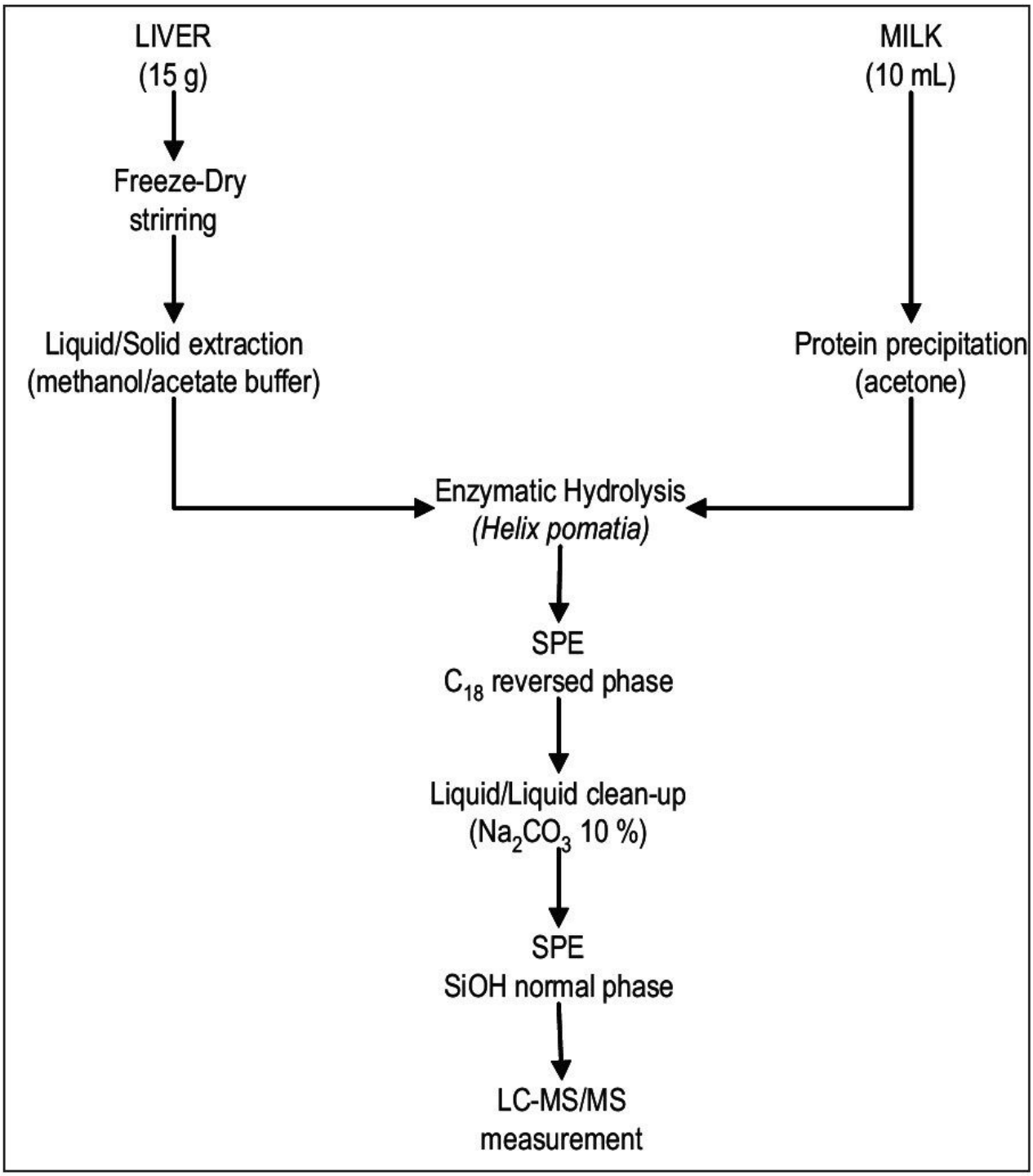

Figure 1. Overview of sample preparation for liver and milk samples.

LC conditions

LC system:	ACQUITY UPLC System
Runtime:	7.0 min
Column:	ACQUITY UPLC BEH C_{18} Column 1.7 µm, 2.1 x 100 mm
Mobile phase A:	0.5% acetic acid dissolved in water
Mobile phase B:	acetonitrile
Flow rate:	0.6 mL/min
Injection volume:	2 µL

MS conditions

MS system:	Xevo TQ MS
Ionization mode:	ESI negative
Capillary voltage:	3 kV
Source temp:	150 °C
Desolvation temp:	500 °C
Desolvation gas:	920 L/H
Collision gas flow:	0.15 mL/min

	Time (min)	Flow rate (mL/min)	%A	%B
1.	Initial	0.6	75	25
2.	4.0	0.6	75	25
3.	4.3	0.6	0	100
4.	5.0	0.6	0	100
5.	5.1	0.6	75	25
6.	7.0	0.6	75	25

MRM method parameters

Diagnostic MRM transitions were first generated using Waters' IntelliStart Technology. All the parameters (detailed in the following table) were then optimized individually for each diagnostic signal.

Compound name	Parent (m/z)	Daughter (m/z)	Dwell (s)	Cone (V)	Collision (eV)
Fludrocortisone (2^{nd} internal standard)	349.2	295.1	0.094	40	22
		313.2	0.094	40	20
Fluorometholone (external standard)	355.2	255.1	0.094	34	14
d4-dexamethasone (1^{st} internal standard)	363.2	309.1	0.094	40	20
		327.2	0.094	40	18
Dexamethasone and betamethasone	451.2	361.2	0.094	20	18
		307.2	0.094	20	30
	361.2	307.2	0.094	40	18
		325.2	0.094	40	20

Results and Discussion

Critical separation of dexamethasone from betamethasone

In order to obtain accurate determinations for these particular growth promoters, it is essential that chromatographic separation of the epimers dexamethasone and betamethasone is achieved. An efficient separation of betamethasone (t_R=3.14 min) from dexamethasone (t_R=3.25 min) was observed using isocratic gradient in the range [0-4 min]. These UPLC conditions allow identification of molecules from their relative retention time (Figure 1).

Regulatory target analysis of dexamethasone and betamethasone in milk

Four diagnostic MRM transitions were set for the identification of dexamethasone and betamethasone in order to fulfil the regulatory requirements of the European Commission Decision 2002/657:EC[7]: these four diagnostic transitions (451 > 361, 361 > 307, 451 > 307, and 361 > 325) were selected in the MRM transition mode in order to perform unambiguous identification of the compounds. Moreover, d_4-dexamethasone (363 > 309, t_R= 3.56 min) was used as internal standard because of its mimetic properties with dexamethasone.

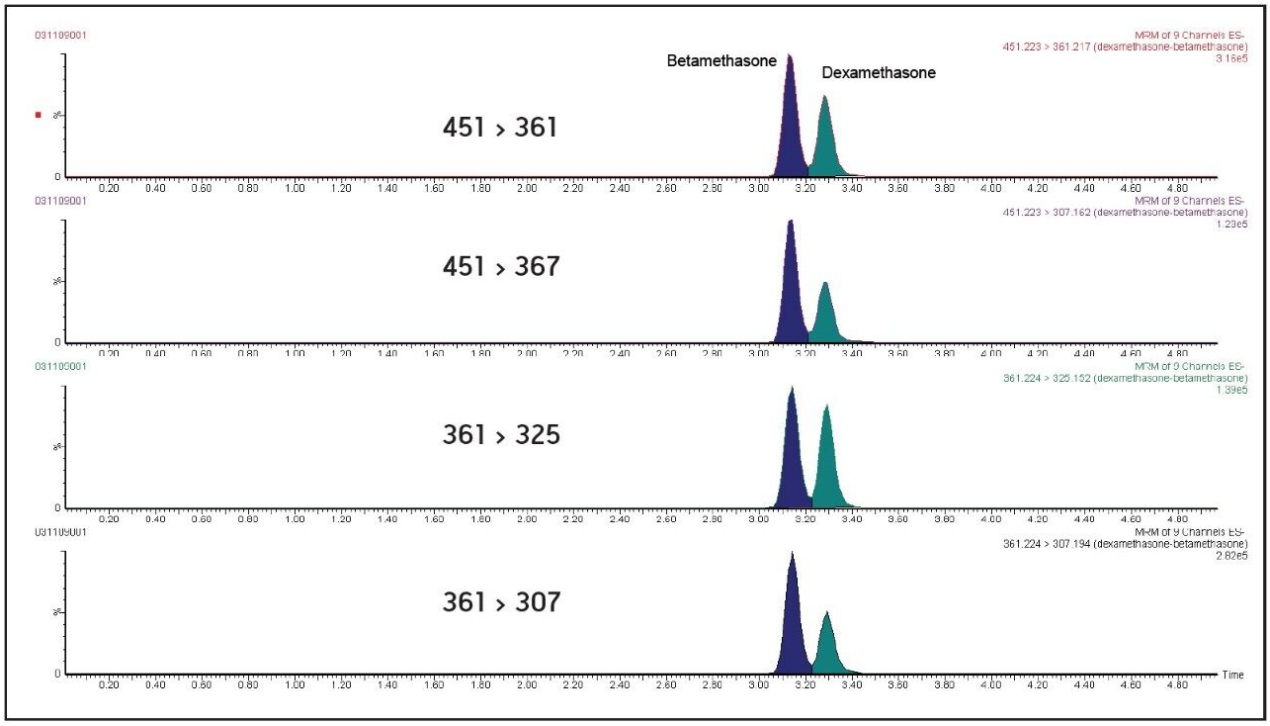

Figure 2. UPLC (ESI-)/MS/MS MRM diagnostic signals of dexamethasone, betamethasone (451 > 361, 361 > 307, 361 > 325, and 451 > 307) obtained from a standard solution at 1 ng/mL.

Blank milk sample chromatograms are shown in Figure 3a with no positive response for dexamethasone and betamethasone at the expected retention time. This demonstrates the selectivity of the methodology with the combination of chromatographic resolution and instrumental selectivity. Extracted MRM chromatograms corresponding to milk samples fortified at 0.3 µg/L (MRL) and 0.075 µg/L (4 times below MRL) are shown in Figures 3b and 3c. High instrument sensitivity allows highly confident identification of both substances to be performed (Identification points=10), even at concentrations that are 4 times below the European MRL.

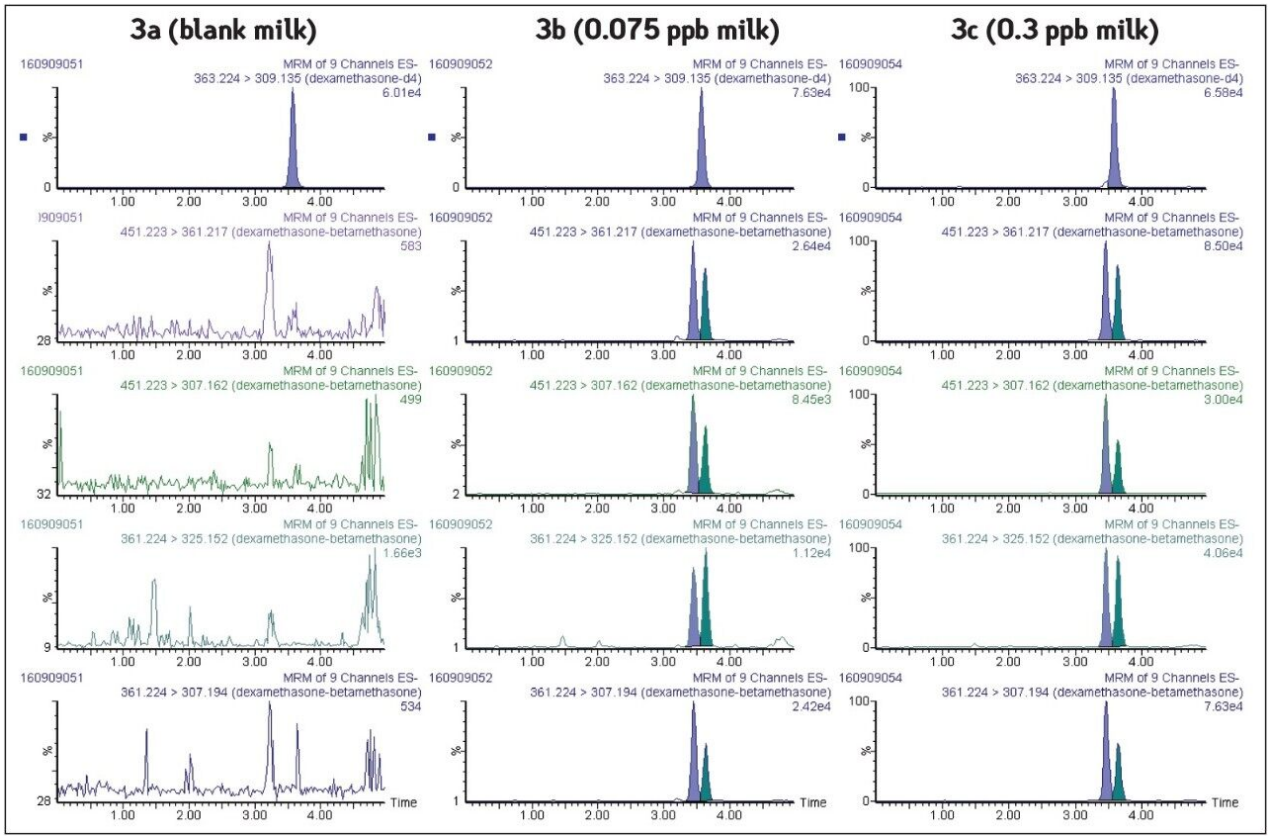

Figure 3. UPLC (ESI-)/MS/MS SRM diagnostic signals of dexamethasone, betamethasone (451 > 361, 361 > 307, 361 > 325 and 451 > 307) and d_4-dexamethasone (363 > 309) obtained from a blank milk sample (3a) and spiked milk samples: 0.075 ppb (3b), and 0.3 ppb (3c).

Regulatory target analysis of dexamethasone in liver

To test performance around the regulatory limits, fortified liver samples were analyzed at twenty times below, four times below, and at the European MRL for dexamethasone (2.0 ppb). The extracted MRM chromatograms for each level are shown in Figure 4. Positive identification according to regulatory requirements (2002/657/EC) was comfortably achieved with 10 identification points (IP=10) for concentrations around the MRL (3 IPs are mandatory with 4 IPs reserved for illegal substances). As with previous determinations in milk, the method selectivity was such that the blank liver samples did not show any response (were s/n > 3).

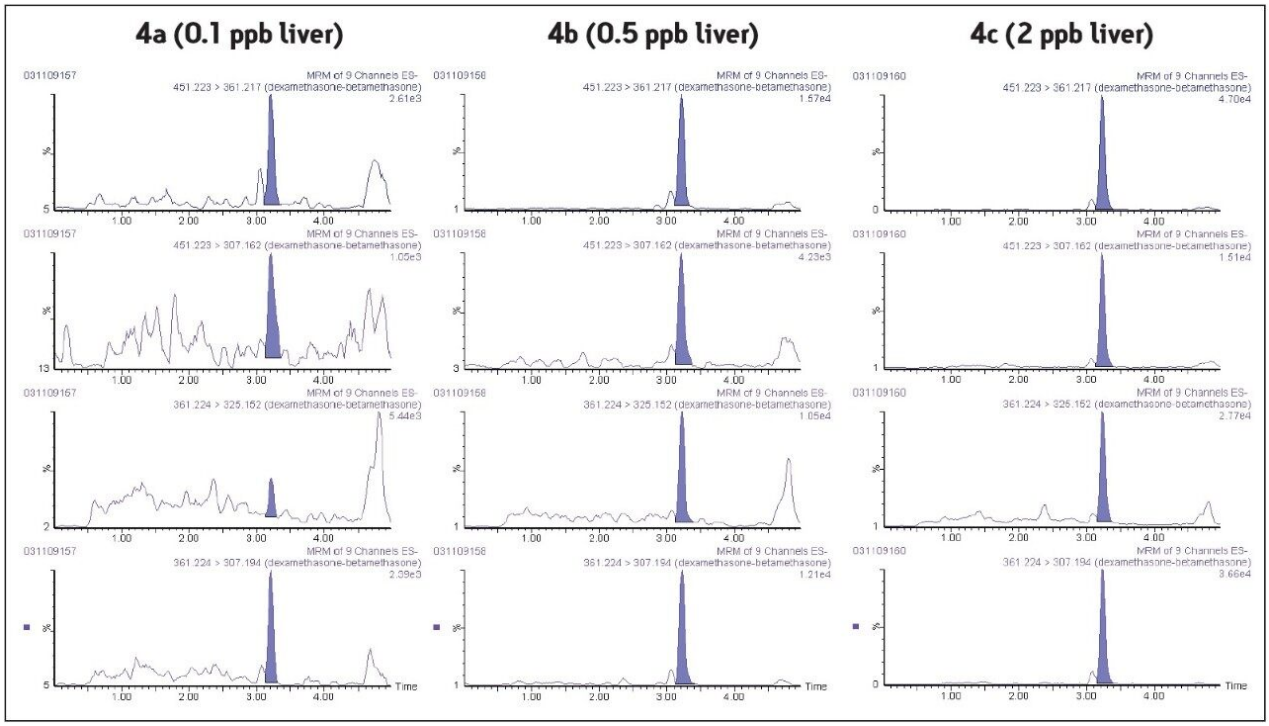

Figure 4. UPLC (ESI-)/MS/MS MRM diagnostic signals of dexamethasone, (451 > 361, 361 > 307, 361 > 325 and 451 > 307) obtained from the following various spiked liver samples: 0.1 ppb (4a), 0.5 ppb (4b), and 2.0 ppb (4c).

Method validation

The stability of the transition ratios was also evaluated in the different matrices for all concentrations. Signals obtained were very repeatable even at very low concentration levels (MRL/4 for milk: 0.075 ppb and MRL/20 for liver: 0.1 ppb) with an unambiguous identification of the compound (IP=10).

Concerning the liver matrix, linearity was evaluated by plotting relative peak height ratios in the range [0 to 10 µg/kg]. The correlation coefficients of the calibration curve were above 0.99. At the MRL level, the relative standard deviations for signal responses were calculated to 6.4% and 6.1% for betamethasone and dexamethasone. At the same level, the relative standard deviations for relative retention times were 0.16% and 0.20% for betamethasone and dexamethasone respectively.

CCα (decision limit) and CCβ (detection limit) were determined in accordance with European Decision 2002/657. CCα were 2.31 and 2.35 while CCβ were 2.57 and 2.63 for dexamethasone and betamethasone respectively.

Additional residue confirmation using Product Ion Confirmation Scan (PICS)

Data directed acquisition allows spectral data to be collected when a particular parameter is detected above a certain threshold. Very rapid switching between quadrupole static mode and scanning mode is essential for this to happen in real time whilst a chromatographic peak is eluting. This capability allows additional information when trying to confirm presence of residues in a sample and is an additional step that can be taken to help identify and investigate false positive results.

Product ion confirmation scan (PICS) allows a product ion scan to be acquired which is triggered by a selected MRM transition reaching a critical threshold. This capability is especially relevant when applied to supporting the identification of a target substance. Figure 5 shows extracted MRM chromatogram for dexamethasone at European MRL in a liver sample which has PICS enabled. Figure 5 also shows the product ion spectrum from the automatically triggered scan from the 361 > 307 transition. This spectrum can then be compared to an MS/MS library to provide additional evidence for presence of dexamethasone.

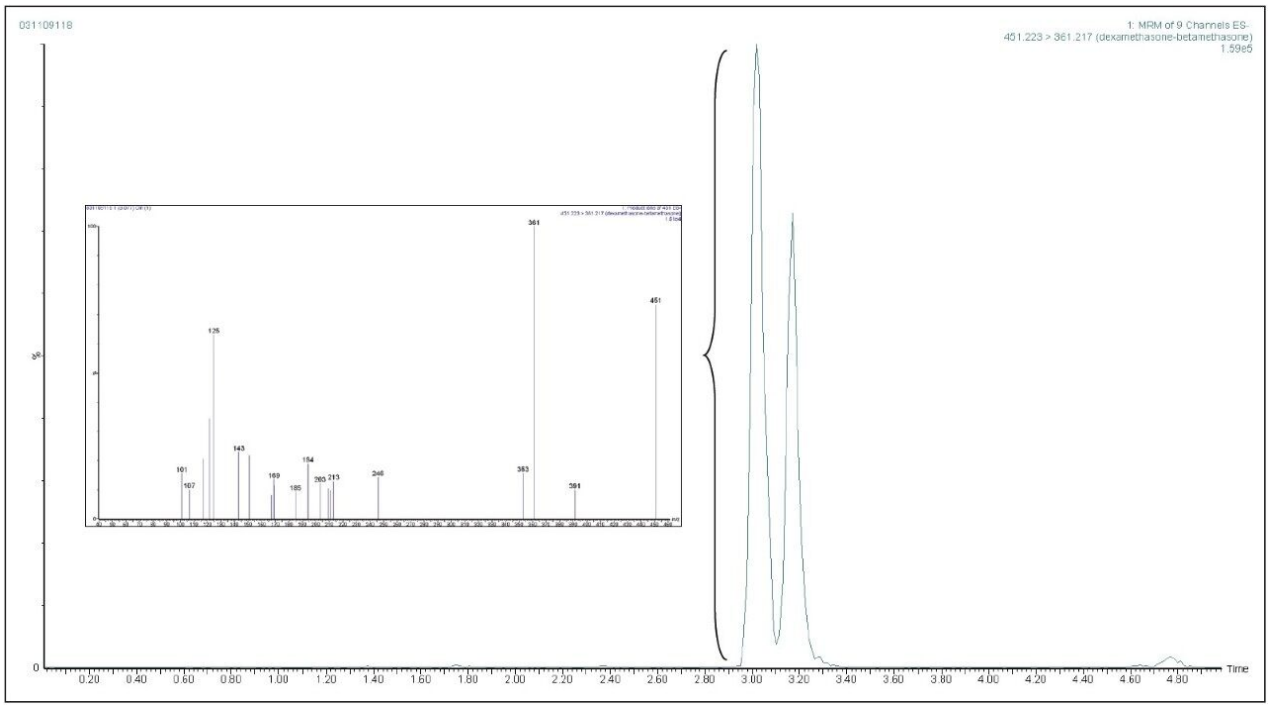

Figure 5. UPLC (ESI-)/MS/MS MRM for dexamethasone (451 > 361) with triggered ScanWave product ion scan spectrum for a liver sample spiked at twice the MRL (4 ppb).

Conclusion

- Xevo TQ MS allowed highly selective and sensitive analysis for dexamethasone and betamethasone in complex matrices.
- European MRLs and confirmation requirements (2002/657/EC) were comfortably achieved using Xevo TQ MS.
- The high chromatographic resolution of the ACQUITY UPLC System facilitated the critical separation of the epimers dexamethasone and betamethasone.
- Additional spectral information can be obtained by data directed PICS which in turn could help confirm the presence of banned substances

References

1. C M Kahn, S Line (Eds), The Merck Veterinary Manual, Anti-inflammatory Agents, 9th ed., Merck and Co Inc., New Jersey, 2005.
2. EEC Council Directive No. 96/23, Off. *J. Eur. Commun.* No. L125, 1996
3. Commission Regulation (EU) No. 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.
4. E M Malone, G Dowling, C T Elliott, D G Kennedy, L Regan*, J Chromatogr. A.* 1216 (2009) 8132-8140.
5. Li Cun, Wu Yinliang, Yang Ting, Zhang Yan, *J Chromatogr. A.* 1217 (2010) 411-414.
6. J P Antignac, B Le Bizec, F Monteau, F Poulain, F Andre, *J Chromatogr.* B 757 (2001) 11.
7. Commission Decision (2002/657/EC) of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and interpretation of results, Off. *J. Eur. Commun*. L221 (2002) 8.

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