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# DEVELOPMENT OF A RAPID AND SENSITIVE LC-MS/MS METHOD FOR THE SIMULTANEOUS IDENTIFICATION AND QUANTITATION OF CORTICOSTEROIDS IN BOVINE LIVER EXTRACTS

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## Introduction

Corticosteroids are used in many veterinary therapeutic drugs for their anti-inflammatory properties. However, they are also illegally used as growth promoters in cattle because these compounds increase the gain in weight of some animals and have a synergethic effect with other growth promoters.

To control the use of these growth promoters a rapid and sensitive method was developed for the analysis of 6 corticosteroids (Triamcinolone Acetonide, Flumethasone, Dexamethasone, Betamethasone, Methylprednisolone and Prednisolone) in bovine liver extracts using liquid chromatography tandem mass spectrometry. Dexamethasone-$d_3$ was used as an internal standard. Considering European Union legislation, special interest was shown into the separate confirmation and quantification of dexamethasone and betamethasone residues in liver extracts. This study was based on an earlier study performed by the laboratory of Prof. C. Van Peteghem[1]. However in this report, compared to the previous study, a wider range of compounds (total of 6 different corticosteroids) was analysed on a higher end instrument (Quattro Ultima™).

## Experimental Conditions

### *Sample Preparation*

The analysed corticosteroid samples were fortified bovine liver extracts around the maximum residue level (MRL). Calibration standards and control (QC) samples were both spiked in the extracts. An overall scheme for the extraction procedure is shown below.

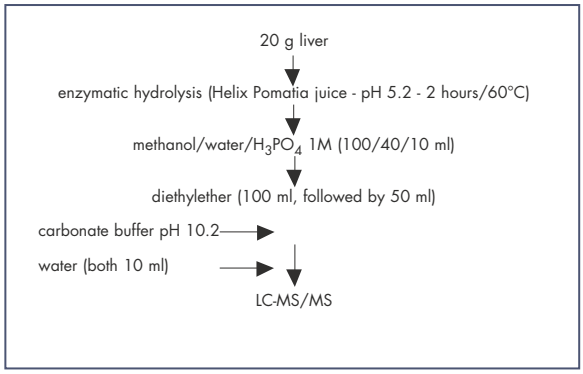


*Figure 1. Overall scheme for the tracing of corticosteroids in liver*

### *LC conditions*

| | |
|---|---|
| HPLC system: | Waters 2790 XE system |
| Columns: | Hypersil Hypercarb (4.6 x 100 mm, particle size 7 µm) |
| Mobile phase: | Water/Acetonitrile, each with 0.5 % acetic acid (10/90, v/v) |
| Flow rate: | 1.5 ml/min (flow was splitted 1/5 before entering the source) |
| Injection Volume: | 10 µl |
| Column Temperature: | 35 °C |
| Chromatographic RunTime: | 15 min |

### *MS conditions*

| | |
|---|---|
| Mass Spectrometer: | Triple quadrupole - Quattro Ultima (Micromass) (picture and schematics, see **Figure 2**) |
| Ionisation Mode: | ES -ve |
| Capillary Voltage: | 3.8 kV |
| Source Temperature: | 120 °C |
| Desolvation Temperature: | 300 °C |

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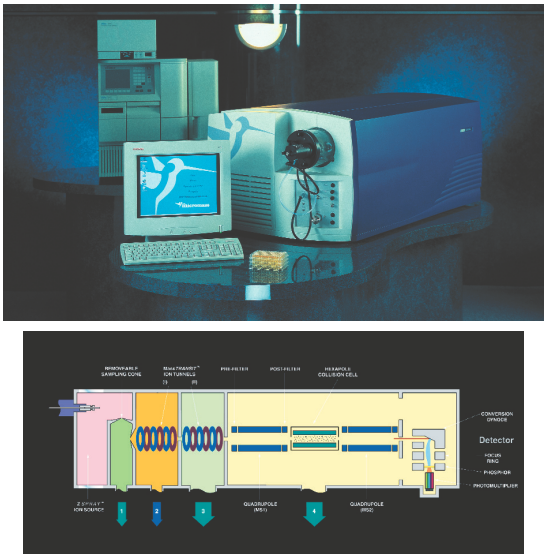


*Figure 2.*

## Results and Discussion

The main aim of the chromatographic development was to separate dexamethasone from betamethasone since those two isomers are, based on their mass spectrometric behaviour, indistinguishable from each other (same protonated molecular ions, as well as product ions). To solve this problem a graphite column (Hypersil Hypercarb - 4.6 x 100 mm, particle size 7 µm) and isocratic elution with water/acetonitrile, each containing 0.5 % acetic acid (10/90, v/v) proved to be successful. The chromatographic separation of betamethasone and dexamethasone allowed us to quantify both compounds separately. **Figure 3** shows the LC-MS/MS chromatograms obtained for the analysis of blank bovine liver fortified at 1 ppb.

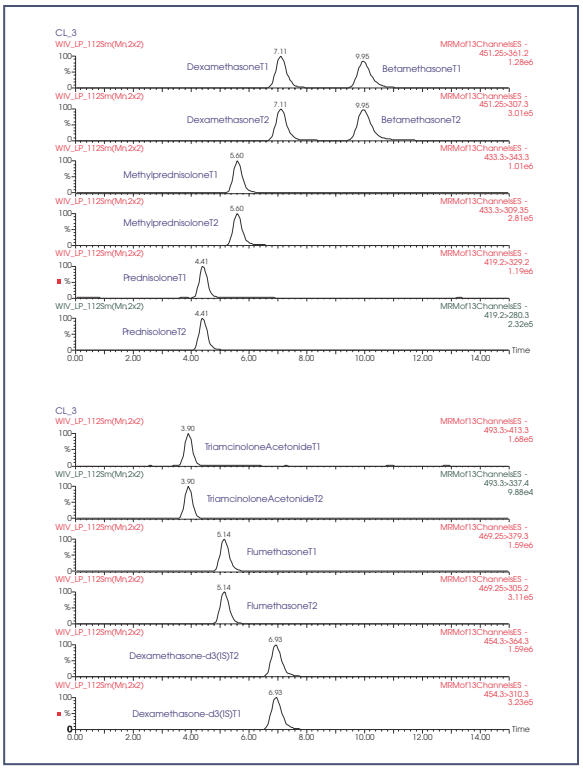


*Figure 3. LC-MS/MS chromatograms of a spiked bovine liver sample (concentration 1 ppb)*

For the quantification and confirmation of the identity of all investigated corticosteroids, two multiple reaction monitoring (MRM) transitions per compound were used (for the transitions see **Table 1**), in compliance with the EU draft recommendations (1 precursor and 2 product ions per residue). The ratio of the MRM transitions can be monitored for positive confirmation.

| COMPOUND | MRL (ppb) | Followed Transition | Cone Voltage | Collision Energy |
|---|---|---|---|---|
| Triamcinolone Acetonide | 2 | 493 > 413 | 40 | 20 |
| | | 493 > 337 | 40 | 25 |
| Flumethasone | 2 | 469 > 379 | 35 | 18 |
| | | 469 > 305 | 35 | 40 |
| Dexamethasone | 2 | 451 > 361 | 40 | 18 |
| | | 451 > 307 | 40 | 32 |
| Betamethasone | 2 | 451 > 361 | 40 | 18 |
| | | 451 > 307 | 40 | 32 |
| Methylprednisolone | 10 | 433 > 343 | 30 | 18 |
| | | 433 > 309 | 30 | 32 |
| Prednisolone | 10 | 419 > 329 | 35 | 18 |
| | | 419 > 280 | 35 | 37 |
| Dexamethasone-d3 (I.S.) | - | 454 > 310 | 40 | 32 |
| | | 454 > 364 | 40 | 18 |

*Table 1. The different MRM transitions which are used for the quantification, together with the applied cone voltage and the used collision energies.*

The developed method proved successful for the quantification of all 6 corticosteroids in liver extracts. Two representative calibration curves (of transition 433>343 (T1) and 433>309 (T2) of methylprednisolone) are shown in **Figure 4**. As can be seen from this figure both transitions showed good linearity. This was the case for all the followed MRM transitions of the 6 measured corticosteroids with coefficients of determination of 0.98 or better (1/x weighted regression) in the concentration range of 0.2 - 20 µg/kg (concentrations used for the calibration curves are: 0.2; 0.5; 1.0; 5.0; 10.0 and 20.0 µg/kg). Considering that the MRL for most corticosteroids in liver samples is 2 µg/kg (**Table 1**), the developed method is able to quantify samples at levels which are more then 10 times below this MRL value.

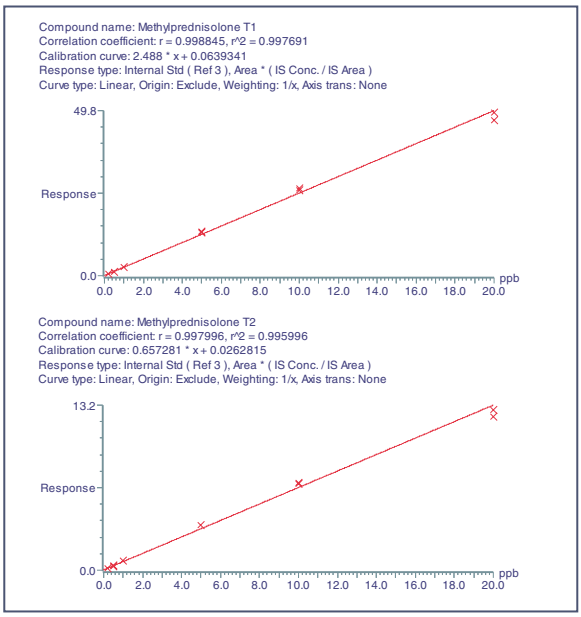


*Figure 4. Calibration curves for methylprednisolone*

The described method was used for the analyses of spiked control samples. To that end, the control samples were analysed in double, the concentrations were calculated and compared with the theoretical added concentrations. The results of this experiment can be found in **Table 2**.

| Spiked Control (QC) Samples | COMPOUND | THEORETICAL CONCENTRATION (PPB) | EXPERIMENTAL CONCENTRATION (PPB) |
|---|---|---|---|
| 1 | Flumethasone | 2 | 2.5 |
| | Prednisolone | 10 | 9.5 |
| | Dexamethasone | 2 | 2.4 |
| 2 | Triamcinolone Acetonide | 2 | 2.2 |
| | Methylprednisolone | 5 | 4.0 |
| | Dexamethasone | 1 | 1.2 |
| | Betamethasone | 2 | 2.0 |

*Table 2. The experimental and theoretical concentrations which were found in spiked bovine liver samples.*

## Conclusion

The described LC-MS/MS method proved to be very efficient for the simultaneous identification and quantification of corticosteroids in liver extracts, including the separate confirmation and quantification of betamethasone and dexamethasone residues at levels more then 10 times below the imposed MRL levels.

## References

1. O. Van den Hauwe, J. Castro Perez, J. Claereboudt, and C. Van Peteghem. *Rapid Commun. Mass Spectrom.* 2001, 15: 857-861.

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