Analysis of selected Steroids and Stilbenes

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Introduction

Steroid hormones have been used worldwide for many years to improve rates of protein deposition in livestock. In spite of the official ban, a black market demand for hormones and hormone cocktails does exist. For controls to be effective at the stall level as well as for imported products in the EU, the development of more sensitive, more adaptable, more specific and more efficient detection methods was necessary. These detection methods must satisfy the requirements of current EU legislation on confirmation criteria, Commission Decision 657/2002/EC.
In this study a multi-residue method is described suitable for the determination of anabolic steroids in multiple matrices like meat, fish and feeding stuff.

Experimental

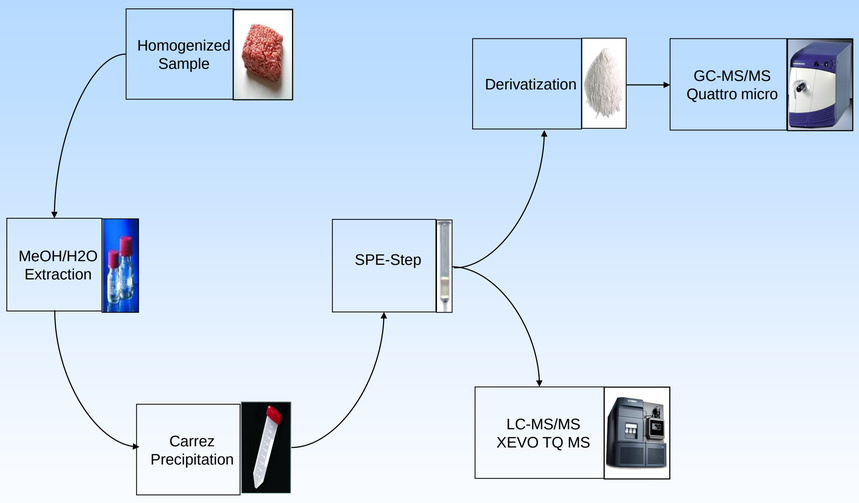

Fig .1 sample preparation scheme

- Extraction is performed with a solvent mixture followed by a MSPD procedure
- Unwanted matrix compounds are eliminated by a Carrez-Precipitation followed by a selective SPE-cleanup
- The final extract is analyzed both:
  - by LC-MS-MS in APCI mode (XEVO TQ MS, Waters)
  - and GC-MS-MS (Quattro micro GC, Waters) after a derivatization step

Tab.2 MS parameters XEVO TQ MS (Waters)

Parameter	m/z Parent	Daughter 1	Daughter 2	Polarity
Trenbolon	271.25	199.34	253.4	+
Nortestosteron	275.30	109.19	81.34	+
Testosteron	289.30	97.29	109.22	+
Methyltestosteron	303.31	97.27	109.17	+

Tab.3 MS parameters Quattro micro GC (Waters)

Parameter	m/z (TMS) Parent	Daughter	Polarity
Hexestrol	207	179	+
	208	180	+
Dienestrol	395	379	+
	395	381	+
Diethylstilbestrol	412	179	+
	412	217	+
17-β-Estradiol	416	285	+
	416	129	+
Zeranol	433	295	-
	538	433	-

Results

- The method was validated according to DIN ISO 32645 and 657/2002/EC
- Validation according to 657/2002/EC was accomplished by using the ‚Software Interval Plus (quo data GmbH)
- The following variations were applied:
  - different kinds of meat
  - variation in the fat content
  - sample preparation by different persons
  - sample storage at different conditions
  - storage of the sample extracts for different times
- All values for cc α and cc β fall below the concentration of 1 ppb
- the results met the requirements of EC legislation

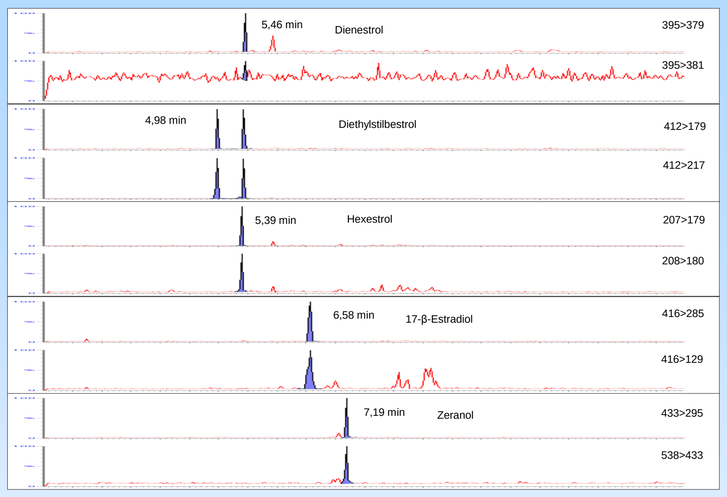

Fig.2 Spiked pork sample 1,5 µg/kg, aquired by GC-MS/MS (Quattro micro GC, Waters)

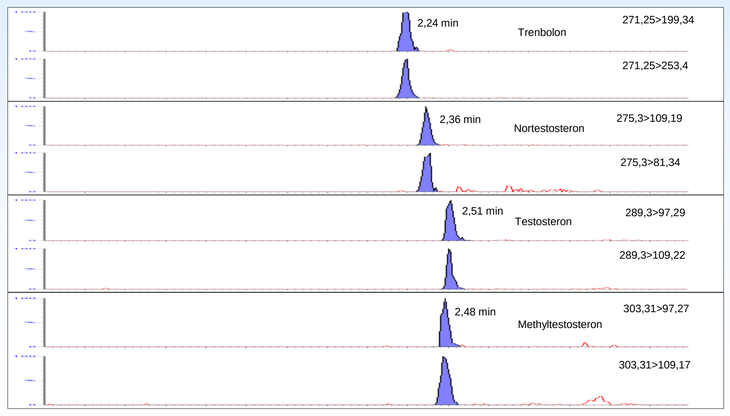

Fig. 3 spiked poultry sample, 1,5 µg/kg, aquired by LC-MS/MS (XEVO TQ MS)

Tab.4 Validation data according to DIN 32645

Parameter	cc α	cc β
Diethylstilbestrol	0,77	0,79
17-β-Estradiol	0,76	0,79
Dienestrol	0,77	0,79
Hexestrol	0,77	0,79
Methyltestosteron	0,82	0,87
Nortestosteron	0,76	0,79
Testosteron	0,77	0,79
Trenbolon	0,77	0,79
Zeranol	0,76	0,79

Conclusion

- A new sensitive, robust and fast method was developed and validated for selected steroids and stilbenes
- This study is a fast and economic procedure to determine a wide scope of compounds
- This method is applicable to many matrices like meat, fish and feeding stuff
- In the near future the analytical procedure will be extended by new parameters and matrices like milk products, urine and faeces