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Improvement of estradiol esters monitoring in bovine hair by dansylation and liquid chromatography/tandem mass spectrometry analysis in multiple reaction monitoring and precursor ion scan modes

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RATIONALE: The control of forbidden anabolic practices in cattle in the European Union has become challenging since endogenous compounds such as estradiol derivatives can potentially be used as growth promoters. Due to the great difficulty in establishing a reference threshold value for endogenous steroids, the direct detection of steroid esters in hair is an efficient strategy for the detection of 'natural' steroid abuse in cattle.

METHODS: The present study aimed to develop and validate according to the current European standards a specific liquid chromatography/tandem mass spectrometry (LC/MS/MS) analytical strategy to monitor estrogen esters in bovine hair. The analysis was performed by positive ion electrospray ionisation (ESI+) after dansylation. Two acquisition modes were then assessed: single reaction monitoring and precursor ion scanning.

RESULTS: The results showed that the introduction of a dansylation step strongly improves the sensitivity of the detection of estradiol-17-esters by LC/(ESI+)-MS/MS. The CCα values are in the range 1–10 ng g^{-1} after optimisation, except for estradiol decanoate for which the derivatisation is not efficient. In addition, this LC/MS/MS approach makes it possible to carry out a precursor ion scan to screen for the presence of these estradiol 17-esters in hair samples.

CONCLUSIONS: Based on the specific product ions, i.e. *m/z* 255 in native conditions or *m/z* 171 after dansylation, this strategy has the advantage of detecting any (un)known estradiol ester and of giving access to the $[M+H]^+$ ion of the suspected ester through only a single analysis.

The use of growth promoters in cattle fattening is prohibited in the European Union according to Directive 96/22/EC.[1] Since the 1990s, the concentration levels of xenobiotics have been decreasing and it seems that steroid misuse is shifting toward natural hormones.[2] Currently, one of the most difficult challenges is probably the differentiation of mimetic compounds from the natural ones, where the suspicious signal is hidden by that arising from the endogenous production of the animal. This is mainly the case for testosterone and estradiol, but it also affects nandrolone and boldenone.

In the last decade, several analytical solutions have been developed to improve the monitoring of these substances, based on an easily available and non-invasive biological matrix, e.g. urine. These analytical strategies are usually based on targeted gas chromatography/tandem mass spectrometry (GC/MS^n) or liquid chromatography/tandem mass spectrometry (LC/MS^n) measurement methods.[3–11] However, finding evidence of the administration of a natural steroid still represents a very difficult challenge, mainly due to the high variability of the endogenous metabolites in urine between animals.[12] As a result, non-ambiguous analytical strategies, also called confirmatory methods, were implemented in control laboratories. As an example, the confirmation of estradiol and testosterone abuse in cattle is performed by measuring the isotopic deviation ($\delta^{13}C_{V\text{-}PDB}$) of these compounds by gas chromatography coupled to combustion isotope ratio mass spectrometry (GC/C/IRMS). In this case, elucidation of the steroid origin provides unambiguous confirmation of illegal administration.[13–15] In parallel, the identification of some markers of administration, never found in non-treated animals, is a powerful alternative method of confirming the misuse of boldenone[16] or nandrolone in cattle.[17] However, the corresponding metabolism has to be completely elucidated before such efficient targeted analytical strategies can be employed. In addition, the use of urine as the biological sample has the drawback that xenobiotics are rapidly eliminated through the excretion of metabolites in urine. Detection sensitivity remains the major issue in these different approaches, reducing the time window of detectability to rarely over one week after administration.

Since the 1990s, several approaches to detect natural steroid hormones administration have been described on sweat extracts, and more widely on hair in drug testing,[18–20] doping control,[21,22] and livestock production monitoring.[6,23] The hair sample presents some advantages, in particular in being a non-invasive sample easy to obtain, but also in allowing a

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longer detection window after administration than urine. Furthermore, in hair, the administered substance is still present under its administered form.

In view of these major advantages, several developments have been carried out to monitor steroid esters in hair either by gas chromatography/tandem mass spectrometry (GC/MS/MS)[24–26] or by liquid chromatography/tandem mass spectrometry (LC/MS/MS).[27] A method inter-comparison demonstrated reasonable equivalence between the LC/MS/MS and GC/MS/MS methods for the detection of estradiol benzoate and nandrolone decanoate.[28] The preparation procedures include a sample size of 100–300 mg of ground hair, with various washing treatments,[29] and an extraction with MeOH, sometimes coupled to a reducing agent such as tris(2-carboxyethyl)phosphine (TCEP). Extracts can then be directly injected[24] or purified on an solid-phase extraction (SPE) C18 cartridge[27,28] or on successive SPE NH2 and C18 cartridges[25] before analysis. The detection of estradiol benzoate, for example, was still possible 15 days after administration, at which time the concentrations had decreased to the decision limit (CCα) values of previously described analytical methods, i.e. 10 ng.g^{-1}.[27,28]

Nevertheless, longer detectability of steroid esters might be expected in a matrix such as hair, and more modern instruments can deliver higher sensitivity. LC/MS/MS analysis does not seem to be the most suitable and sensitive method for steroid determination, and GC/MS is often preferred. Indeed, the ionisation efficiencies of such compounds in electrospray ionisation (ESI) are actually very low because these substances are not highly polar or even non-polar. Therefore, derivatisation reactions have often been recommended, particularly for estrogen compounds[30–32] with dansylation of ethinylestradiol, or with 2-fluoro-1-methylpyridinium-*p*-toluenesulfonate (FMPTS) and pentafluorobenzyl bromide (PFBBr) derivatives for estradiol, estrone and estriol.[33] The dansylation reaction has already been successfully applied to ethinylestradiol monitoring in hair[34] and in cerebrospinal samples.[35] The use of atmospheric pressure photoionisation was also tested[36] and the results were equivalent to those obtained with ESI + with a sensitivity of detection close to 1 ng.mL^{-1} (lowest calibration point on plasma samples).

Based on the experience acquired for steroid analysis, the aim of the present study was to enhance steroid ester monitoring in hair samples using LC/MS/MS. To this end, the derivatisation reaction was adapted for the first time to estradiol-17-esters, the acquisition parameters were defined and the entire analytical methodology was validated according to 2002/657/EC analytical criteria. The time window of detectability after the administration of estradiol benzoate was determined and compared with published results. In addition, the feasibility of a wider screening tool for the monitoring of estradiol esters in hair was studied based on the specific product ions formed in the MS/MS study of the dansylated derivatives.

EXPERIMENTAL

Chemicals

The reference steroids (listed in Table 1) were from Steraloids Inc. Ltd. (London, UK), Sigma–Aldrich (St. Quentin Fallavier, France) and RIKILT (Wageningen, The Netherlands). Most of the reagents and solvents were of analytical grade quality and provided by VWR International (Pessac, France) and Carlo-Erba Reagents (Rodano, Italy). The SPE columns were from Carlo-Erba Reagents (silica: 1 g, aminopropyl: 0.5 g). The derivatisation reagent (dimethylamino)naphthalene-1-sulfonyl chloride (dansyl chloride) was purchased from Fisher Bioblock Scientific (Illkirch, France).

Table 1. LC/MS/MS parameters used for detecting and identifying each target compound

	Transition (*m/z*)	CV (V)	CE (eV)	Transition (*m/z*)	CV (V)	CE (eV)
norethindrone acetate (IS)	341.2 > 281.2	30	15			
norgestrel (ES)	313.2 > 245.2	30	15			
estradiol-d3 (ES)*	509.2 > 170.8	45	40			
ethinylestradiol (IS)*	530.2 > 170.8	45	40			
stanolone benzoate (IS)	395.2 > 105	30	25			
estradiol-17-benzoate	377.2 > 105.1	30	20	377.2 > 135.1	30	15
estradiol-17-benzoate*	506.1 > 155.8	70	60	506.1 > 170.8	70	40
estradiol-17-cypionate	397.2 > 255.2	25	8	255.2 > 159.1	30	20
estradiol-17-cypionate*	630.3 > 170.8	70	60	630.3 > 155.8	70	40
estradiol-17-decanoate	255.2 > 133.1	30	20	255.2 > 159.1	30	20
estradiol-17-decanoate*	660.3 > 170.8	70	60	660.3 > 155.8	70	40
estradiol-17-enanthate	385.2 > 255.1	25	15	255.2 > 159.1	30	20
estradiol-17-enanthate*	618.2 > 170.8	70	60	618.2 > 155.8	70	40
estradiol-17-propionate	255.2 > 133.1	30	20	255.2 > 159.1	30	20
estradiol-17-propionate*	562.2 > 155.8	50	60	562.2 > 170.8	50	40
estradiol-17-valerate	255.2 > 133.1	30	20	255.2 > 159.1	30	20
estradiol-17-valerate*	590.2 > 170.8	70	60	590.2 > 155.8	70	40
estradiol-3-benzoate	377.2 > 105.1	30	20	377.2 > 135.1	30	15

*Dansylated compound for LC/MS/MS analysis
CV: cone voltage; CE collision energy

Sample preparation

A 100 mg hair sample was ground for about 15 min, sonicated for 1 h with 5 mL of methanol and incubated overnight at 50 °C. After centrifugation, the methanolic extract was removed, evaporated and prepared for successive purifications on NH_2 and SiOH cartridges.[25] The purified extract was then reconstituted in 50 µL of a derivatisation reagent made up of 10 mg dansyl chloride diluted in 10 mL of acetonitrile (ACN)/Na_2CO_3 (10 mM) (50:50, v/v) and held at 60 °C for 40 min. The dansylated steroids were extracted in hexane after liquid/liquid extraction with 1.5 mL hexane against 1.5 mL water. The organic solvent was then evaporated and the final extract was reconstituted in 50 µL of an ACN/H_2O mixture (50:50, v/v).

LC/MS/MS system

An Acquity UPLC® system (Waters, Milford, MA, USA) was used to perform reversed-phase liquid chromatography on a C_{18} and a phenyl column (Acquity BEH C_{18} and Acquity BEH Phenyl, 1.7 µm, 2.1 × 100 mm) at 50 °C. The elution solvents were 0.1% formic acid in H_2O (A) and 0.1% formic acid in ACN (B). The mobile phase composition was 30:70 A/B (v/v): between 0 and 2 min, 0:100 from 8 to 9 min, and back to the initial conditions until 12 min have elapsed. The flow rate was 600 µL min^{-1}. The injection volume was 5 µL. Data were acquired on a triple quadrupole XEVO-TQMS mass spectrometer (Waters) operating in positive electrospray ionisation (ESI+) mode. Nitrogen was used as the desolvation gas at a flow rate of above 900 $L.h^{-1}$. The source and the desolvation temperatures were set at 150 and 450 °C, respectively. The capillary potential was set at 3.5 kV. Argon was used as the collision gas at a flow rate of 0.15 mL min^{-1} for Multiple Reaction Monitoring (MRM) and Precursor ion Scan (PS) experiments. For MRM acquisition, the collision energies and cone voltages were optimised for each compound (Table 1). For the PS mode, the cone voltage, collision energy, scan range and product ions were respectively 30 V, 15 eV, from *m/z* 254 to 500 and *m/z* 255 for underivatised estradiol esters, and 70 V, 50 eV, from *m/z* 500 to 670 and *m/z* 171 for dansylated estradiol esters. For these optimisations, each standard was directly introduced at a concentration of 10 ng/µL in H_2O/ACN/formic acid (50:50:0.1, v/v/v) with a flow rate of 10 µL/min. At this flow rate, the desolvation temperature and gas flow were, respectively, decreased to 300 °C and 600 $L\,h^{-1}$. TargetLynx (Waters) software was used for the integration and assignment of all chromatographic peaks acquired in MRM mode. All the analytes were identified according to the Decision 2002/657/EC,[37] on the basis of retention times and transition ratios.

Validation protocol

The validation procedure was based on Decision 2002/657/EC.[37] We analysed 20 blank hair samples (free of estrogen esters) collected from porcine and bovine species, exhibiting various colors, from dark to white. One sample pool of all the samples was also used to build the calibration curve on sample extracts for each batch extracted (6 batches in the global validation extracted on 6 different days and spiked from 2.5 to 200 ng/g). The decision limit (CCα) was determined with the calibration curve from the average of the noise intensity for the 20 samples at the retention time of the analyte + 2.33 times the associated standard deviation. The detection capability (CCβ) was calculated from the intensity value of CCα + 1.64 times the standard deviation of the within-laboratory reproducibility on each level of the calibration curve.

RESULTS AND DISCUSSION

Optimisation of ESI + MS acquisition parameters

After a brief study in negative ionisation mode, theoretically more appropriated to the ionisation of estrogens, due to their phenol moiety, the positive mode was selected for this study. Under these conditions, the protonated molecule, the $[M+H]^+$ ion, was always detected for all the compounds considered, at a better sensitivity than was obtained for the compounds in negative ion mode (data not shown).

First, an acquisition in SCAN mode was carried out after optimising the capillary and cone voltages to reach the best intensity for the $[M+H]^+$ ion. Then, a product ion scan of the $[M+H]^+$ ion revealed the major product ions associated to a specific collision energy applied in the T-wave collision cell. After a manual ramp of collision energies, two collision energy values were selected per compound, at which product ion scans were recorded. The product ion mass spectra are presented in Fig. 1, for each of the seven studied estradiol esters. Finally, an MRM method was created with at least two diagnostic transitions per compound (cf. Table 1).

PS acquisition mode strategy to screen for estradiol esters

For all the estrogen esters considered, the *m/z* 255 product ion is present from the lowest collision energies (from 8 to 25 eV) (cf. Fig. 1). This ion is specific to the steroid considered, i.e. estradiol here, and represents $[M_{\text{free steroid}} + H - H_2O]^+$. Nevertheless, *m/z* 255 is not the base peak of the estradiol benzoate product ion spectrum, where the main fragmentation pathway leads to the benzoyl ion at *m/z* 105. As the *m/z* 255 ion is characteristic of the estradiol-ester fragmentation, a screening strategy based on this specific ion can easily be set up to monitor estradiol esters in a very specific manner. Therefore, the precursor ion scan of *m/z* 255 was tested. Spiked (between 10 and 25 $ng.g^{-1}$ of all the estrogen esters considered) and blank hair samples were then studied by LC/MS/MS (data not shown). The performance of this method was found to be limited because of the wide variations in the intensity of the *m/z* 255 ion for the seven compounds of interest and because of the presence of a range of interferences which complicated the interpretation. Very poor signal-to-noise ratios were obtained, probably due to a huge matrix effect on *m/z* 255 associated with an acquisition mode which was not sufficiently sensitive. Nevertheless, this strategy is theoretically very promising and it could be implemented with some modifications (see § Precursor ion scan mode for a screening method of (un)known steroid esters).

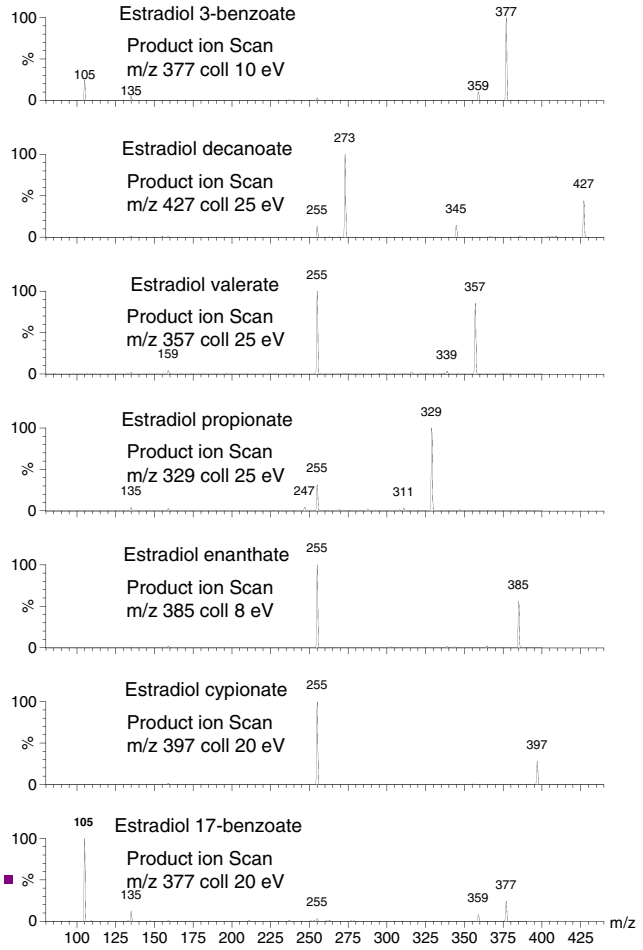

Figure 1. Product ion spectra obtained after fragmentation of each $[M+H]^+$ ion of estrogen esters in (ESI+)-MS/MS.

Derivatisation as a strategy to enhance performance in the detection of estradiol-17-esters

Since a dansyl chloride reagent has been widely used to enhance the detection of estrogens in several matrices,[33–36] it was decided to study its ability to improve the detection of estrogen esters in the present work. The first assay was carried out on standard solutions according to the protocol of Li *et al.*[31] The comparison of all the results was based on the dansylation recoveries to determine the derivatisation efficiency. In LC/(ESI+)-MS/MS, the [dansylated steroid $+H]^+$ ion was always formed in the interface and yielded, after collision, the specific dansyl product ions *m/z* 171 and 156 (loss of a methyl group from the dansyl moiety)[30–32] (cf. Fig. 2). Hence, two transitions were then selected for each of the dansylated estrogen ester studied, from the $[M+H]^+$ ion to the two product ions (cf. Table 1). Furthermore, the dansyl-estradiol esters were stored for several days in the fridge and reinjected without loss of sensitivity, thus demonstrating the stability of these derivatised compounds. (Fig. 3)

The stability of the reagent was investigated because a variation in color between samples and series (more or less yellow in color) was sometimes observed. Several derivatisation mixtures (dansyl chloride/sodium carbonate/acetonitrile) were prepared to test the duration of the reactivity of the reagent, over a week (D7), a full day (D2) and only several minutes before use (D0). The derivatisation was performed for 10 min at 60 °C on all the standards of interest, each derivative solution being tested in triplicate. The dansylation recoveries were calculated in reporting each relative intensity (analyte to internal standard) to the highest one obtained during these assays. These recoveries (Fig. 4) appeared to be linked to the day when the derivatisation reagent was prepared, with an efficient recovery obtained on D0. This led to the conclusion that the derivatisation reagent has to be recently prepared to obtain the best recoveries.

Dansylation does not, however, seem to be suitable for estradiol-17-decanoate, which gave recoveries below 50% (not reported on Fig. 4). Fortunately, the estradiol-3-benzoate derivative is not affected and degraded by the reaction, even when the reagent is most active at D0.

The reaction time was also optimised with the testing, in triplicate, of six different reaction times. The dansylated solution was added to the estrogen ester mixture and held at 60 °C for 0 min, 10 min, 15 min, 20 min, 30 min, 45 min and 60 min. The dansylation recoveries are presented in Fig. 5, calculated in the same way as for the recoveries linked to the reagent preparation. As a first observation, the contact time seems relatively well correlated with the recovery of dansylation. Moreover, for estradiol decanoate, the maximum contact time is required to obtain sufficient recovery yield.

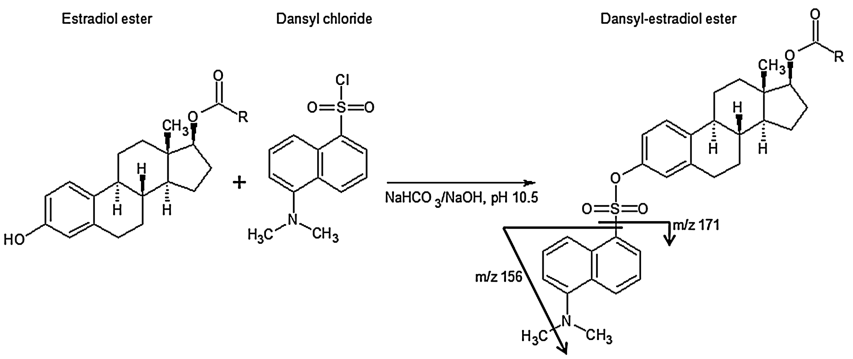

Figure 2. Scheme of dansyl choride derivatisation reaction on estradiol esters (R is an alkyl group) and illustration of the MS fragmentation of the protonated dansyl-estradiol ester with the resulting two product ions.

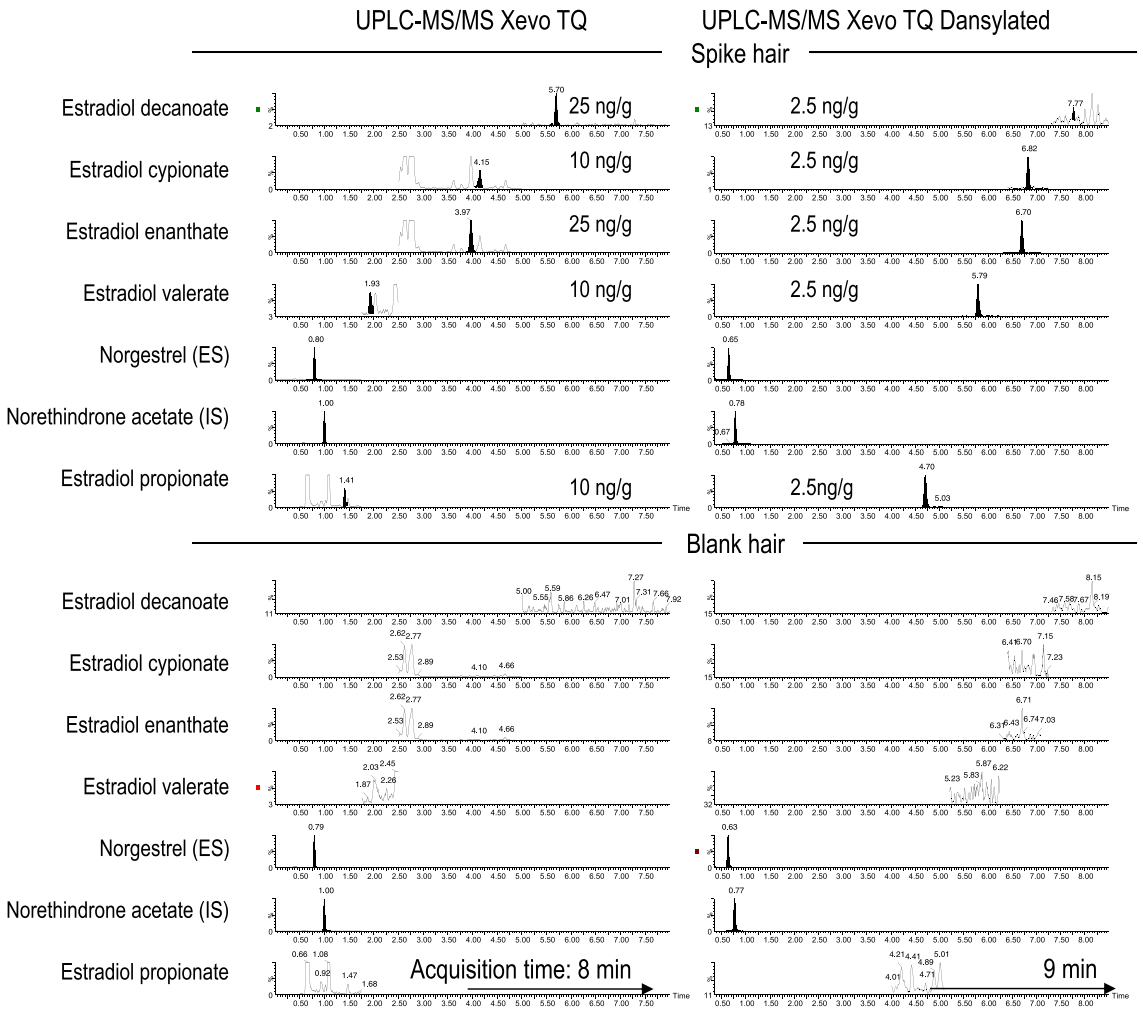

Figure 3. Typical diagnostic chromatograms obtained for estradiol esters in LC/MS/MS in a spiked hair sample (top) and a blank hair sample (bottom) in their native form (without derivatisation, left) and after dansylation (right).

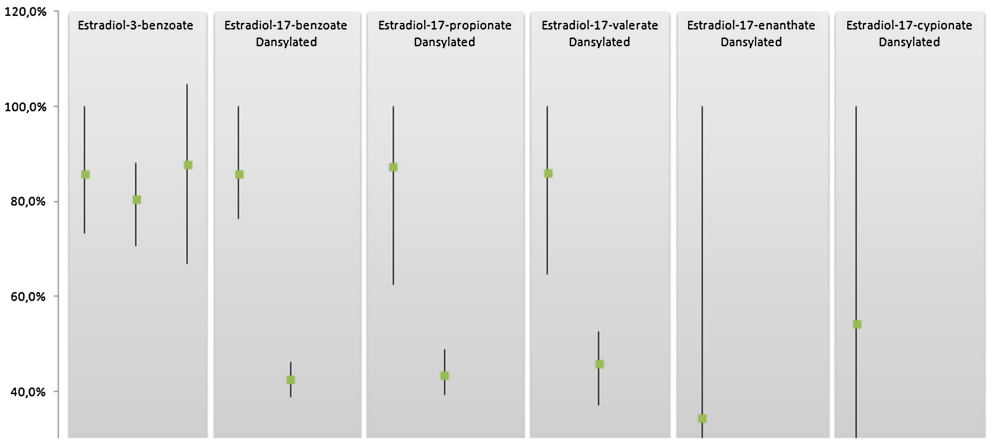

Figure 4. Recoveries yield obtained for the dansylation derivatisation reaction (n = 3) for five estradiol-17-esters derivatised with a reagent prepared on the day of derivatisation (0), 2 days before (2) and 7 days before (7). Estradiol-3-benzoate was also introduced into the mixture to control its stability (the average is represented by a square and the min-max difference by a vertical line).

Thus, the final protocol was to hold the sample at 60 °C for 40 min, to guarantee a sufficient recovery of derivatisation (above 30%) for all the estrogen esters within an acceptable time compatible with routine analysis requirements.

Finally, to control the derivatisation step, several potential internal standards were tested, among which were D_3-estradiol and ethinylestradiol. The best choice would have been an isotopic dilution of estrogen esters, but this type of compound

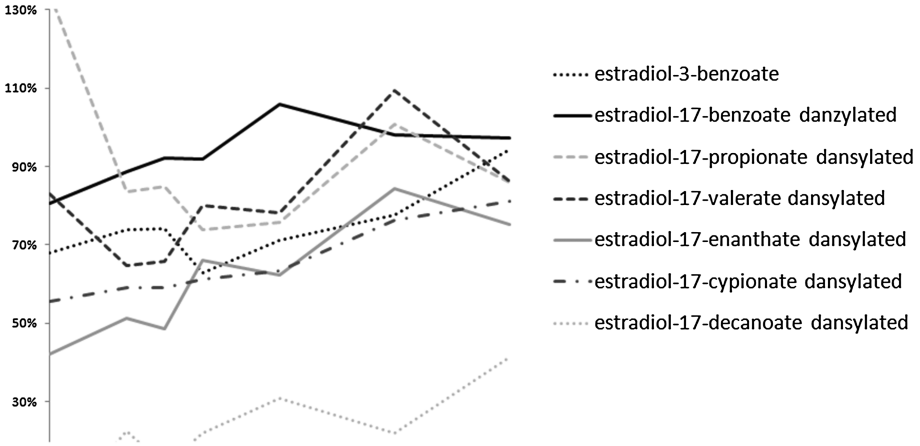

Figure 5. Recoveries of dansylation for six estradiol-17-esters derivatised at 60 °C with a contact time from 0 to 60 min (three replicates per point). Estradiol-3-benzoate was introduced as non-reactive analyte.

is not commercially available. The results showed that only ethinylestradiol presented the same behavior as the steroid esters, with similar recoveries after the extract purification (not shown). Thus, ethinylestradiol was selected as an additional internal standard, able to follow all the analytical process, and D_3-estradiol as an external standard to monitor only the derivatisation step.

Subsequently, derivatisation was tested on spiked samples (cf. Fig. 3). First, the internal and external standards, respectively norethindrone acetate and norgestrel, were not affected by the dansylation and kept a very good peak shape with the same sensitivity (S/N) as without derivatisation. Secondly, the specificity was strongly improved for the majority of the steroid esters after dansylation, allowing easy detection from a sample concentration of 2.5 ng g^{-1}, with a good S/N, as it is shown for estradiol propionate, valerate, enanthate and cypionate (cf. Fig. 3). Rambaud *et al.*[25] obtained estradiol-benzoate concentrations below 20 ng g^{-1} 10 days after administration. Thus, improved sensitivity of detection of such a compound could be obtained by this protocol. Even if the selected product ions in the MRM mode are specific to the dansyl moiety, i.e. *m/z* 171 and 156, the chromatogram for the dansylated fraction appeared the most relevant in term of sensitivity and specificity. However, estradiol-decanoate remains the exception, showing its best sensitivity in LC/MS/MS analysis without derivatisation. Because of this low dansylation yield, estradiol-decanoate might be better monitored with the non-phenol-steroid esters.

Validation results

The CCα and CCβ values are presented in Table 2. The CCα values appear more homogenous for the esters of interest (from 1 to 10 ng g^{-1}) for the LC/MS/MS method if estradiol-17-decanoate is not taken into account. Based on the results shown in Fig. 3 for this latter compound as well as the relatively poor results obtained for the derivatisation recoveries, an analysis of this compound under its native form is recommended. The linearity results were obtained from calibration curves built in the range 2.5–200 ng g^{-1} with at least 10 spiked samples.

Table 2. Decision limit (CCα) and detection capability (CCβ) (in ng g^{-1}) of estradiol esters in hair samples calculated from 20 different blank samples and from the standard deviation of the within-laboratory reproducibility on spike samples. Slope, intercept and determination coefficient for each calibration curve built from at least 10 spike samples (2.5–200 ng g^{-1})

	Transition 1					Transition 2				
	CCα (ppb)	CCβ (ppb)	a	b	R^2	CCα (ppb)	CCβ (ppb)	a	b	R^2
estradiol-17-benzoate*	2.58	9	0.001	0.006	0.766	3	5	0.001	0.002	0.933
estradiol-17-cypionate*	6	51	0.001	0.000	0.995	7	54	0.001	0.000	0.997
estradiol-17-decanoate*	139	189	0.013	−0.086	0.936	243	287	0.007	−0.039	0.930
estradiol-17-enanthate*	1	11	0.014	−0.011	0.962	1	12	0.008	−0.008	0.957
estradiol-17-propionate*	1	5	0.148	−0.039	0.992	1	5	0.095	−0.028	0.993
estradiol-17-valerate*	1	4	0.001	0.000	0.995	1	3	0.007	0.000	0.997
estradiol-3-benzoate	1	1	0.024	−0.008	0.999	7	19	0.002	0.001	0.993

*Dansylated compound for LC/MS/MS analysis

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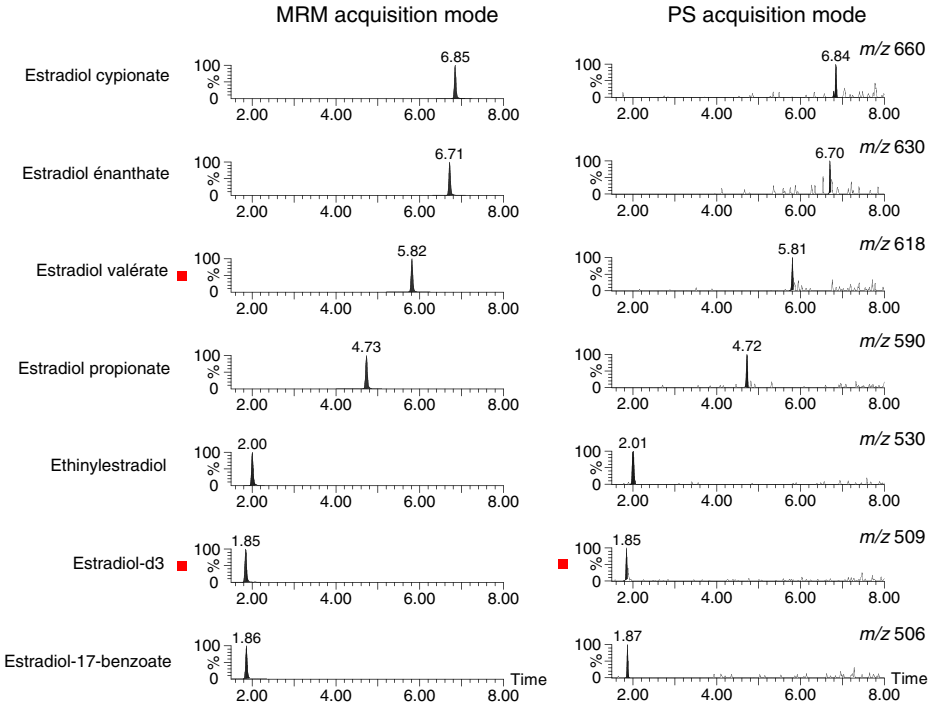

Figure 6. MRM (left) versus PS extracted chromatogram (right) using the Xevo TQMS mass spectrometer for estradiol-17-esters, estradiol-d3 and ethinylestradiol (0.2 ng injected).

PS mode for a screening method of (un)known steroid esters

The screening of unknown steroid esters was then investigated using the precursor ion scan (PS) acquisition mode and improved with the derivatisation step as described above.

The development of this PS method was based on the *m/z* 171 ion which is specific to the dansylated group. At first, detection in MRM mode was compared with that in PS mode on an injected 0.2 ng standard mixture (Fig. 6). As expected, the sensitivity is lower in PS mode and estradiol-decanoate was not detected at this level (not shown). However, all the other estradiol-17-esters were detected when the specific $[M+H]^+$ precursor ion of each compound of interest was selected. Moreover, the internal and external standards, i.e. ethinylestradiol and estradiol-d3, were also detected in this acquisition mode, the recommendation of the EU criteria being respected (Decision 2002/657/EC).

After these first results obtained on standard solutions, a sample obtained from the French national control plan was analysed. Internal and external standards were added at an equivalent of 0.75 ppb in the hair sample. The chromatogram and the associated unknown spectra are presented in Fig. 7. Estradiol-d3 and ethinylestradiol were easily detected in the hair sample, as for the standard. Five other compounds were also detected from which the precursor ion of interest could be extracted. Additional investigations have to be led by the analyst at this stage to confirm the origin of these signals, i. e. comparison with the blank samples, and comparison with other standard spectra. In this case, these precursor ions were not identified as belonging to previously known steroid esters. Therefore, this method appears to be a very interesting tool for studying the wide range of residues monitored, and potentially identifying new estradiol-17-esters. In parallel, the $[M+H]^+$ ion is provided, allowing us to determine the chemical formula of each compound. However, this kind of result requires a significant time for manual interpretation.

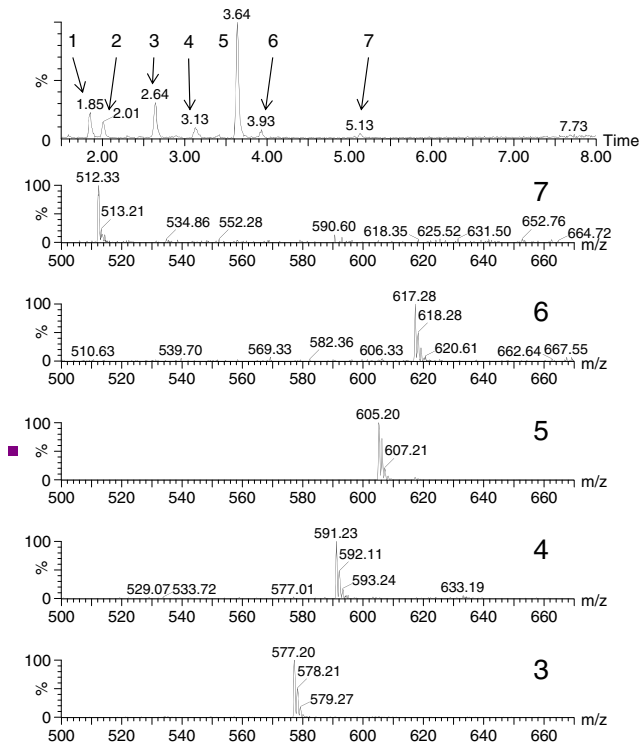

Figure 7. Unknown hair total ion current (top) in PS mode (from *m/z* 500 to 675; precursor ion scan of *m/z* 171). 1: estradiol-d3 dansylated, 2: ethinylestradiol, and associated mass spectra from 3 to 7 retention times (unknown compounds).

An efficient solution to help the analyst would be a new feature of the software where, when a precursor ion of interest is detected above a threshold limit, the instrument carries out an MRM acquisition under the same conditions as the PS acquisition (i.e. energy in the collision cell, product ion to monitor) during at least the peak width time. This protocol would allow improvements in terms of sensitivity and analyst comfort, and also in screening efficiency. Such a mode would make it possible to discard a major part of the false compliant samples in detecting many of the estrogen-17-esters.

In parallel, the yeast bioassays described by Becue *et al.*[38] could be compared with our present screening method. The main advantage of the precursor ion scan method would be the direct access not only to the molecular mass of the ester, but also to that of its native steroid. Currently, the main drawback is that this approach is not ready for use in an extended routine analysis because of the processing difficulties experienced by the operator. A development in the software would be helpful to extract the significant precursor ions from the noise observed for each total ion current (TIC).

CONCLUSIONS

Due to the lack of a threshold value for endogenous steroids in breeding animals, the detection of steroid esters in hair can be considered as one of the key pieces of information needed to detect the synthetic origin of the administered parent compound. A suitable method for the analysis of estrogen-17-esters in bovine hair based on LC/MS/MS has been developed. This method has been validated and it has been shown that a significant improvement in sensitivity can be obtained by the introduction of a derivatisation reagent for the phenol moiety. Furthermore, a new tool could be developed to screen for the presence of hormone esters in hair samples. Even if a software improvement is required, a precursor ion scan method could be adapted to carry out a large screening. Based on the specific product ion of the steroid considered, this strategy presents the advantage of detecting any (un)known steroid ester and giving access to the $[M+H]^+$ ion of the considered ester in only a single analysis.

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