Detection of corticosteroids in injection sites and cocktails by MS^n†

The Analyst

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In the European Union, the use of growth promoting substances such as thyreostats, anabolics (products with estrogenic, androgenic or gestagenic action) and beta-agonists in animal fattening is forbidden. Corticosteroids, such as dexamethasone, although considered catabolic substances, have been administered to food producing animals in order to achieve mass gains. For the analysis of injection sites and of suspect cocktails (found at the farm), a number of HPTLC and HPLC methods are used. However, in injection sites and also in cocktails found at the farm, sometimes many unknown substances are found. In this investigation, a multiple mass spectrometric (MS^n) method was developed. The method is based on rapid extraction of the matrix with methanol and direct infusion of the extract into the interface of the mass spectrometer. Tables that summarise the masses of corticosteroids and their possible esters are presented.

1. Introduction

In Europe, public opinion rejects the use of growth promoters in animal fattening. Increasing surveillance by the inspection services (Belgian Veterinary Food Inspection and Ministry of Agriculture) has resulted in a decrease in the use of the drugs for which adequate analytical methods were available. The use of thyreostats, stilbenes, most anabolic steroids and beta-agonists has decreased considerably or even vanished. However, the so-called "hormone Mafia" discovered that the use of corticosteroids (CoST) in animal fattening could lead to a substantial profit. At first sight and/or from a pharmacological point of view, this was a surprise because CoST are catabolic agents and their use in animal fattening is contra-indicated. However, in practice and also in the literature, indications of the growth promoting effect of corticosteroids was found.[1–4] Also in sports (e.g. pigeon racing) CoST are abused.[5]

At first, the most important CoST abused was dexamethasone (Dxm). Later, other substances such as betamethasone (Btm), triamcinolone (Trm) and/or their esters were detected in injection sites, preparations (cocktails) and animal feed. The consumption of these highly contaminated injection sites (mainly in minced meat) can be a considerable threat to human health or interfere with doping control.[6] Moreover, the administration of these drugs to animals may result in a decrease in animal welfare.

In Belgium, high performance thin layer chromatographic (HPTLC) methods are used for the screening and detection of CoST in injection sites and other matrices containing substantial amounts of CoST.[7–9] These methods are adequate for the identification of substantial amounts of target CoST. "Unknown" TLC spots or "unknown" HPLC peaks are sometimes observed when analysing these matrices.

These unidentified responses to the standard operating procedure (SOP) may be due to interferences from the matrix but also to "unknown" growth promoting substances. In some cases, these "unknowns" may interfere with "target" components. Therefore, it is obligatory that suspect samples should be confirmed by spectrometric techniques.

A gas chromatographic-mass spectrometric method with negative chemical ionisation (GC-NCI-MS) for the detection of Dxm in urine or faeces of treated animals has been described,[10] and other GC-MS methods have been published.[11–13] They provide good sensitivity in analysing samples with low concentrations of analytes but all require time consuming derivatisations which change the structure and also the molecular mass of the molecule. When trying to identify an unknown molecule, it is easier to have a pseudo-molecular species (e.g. MH^+) to start with. LC-MS methods have also been reported.[14–16] The relationship between an observed signal in GC-MS or LC-MS and an "unknown" spot in HPTLC is not always unequivocal (derivatisation in GC-MS may change the molecular mass; a different phase in LC-MS to that in HPTLC may result in a different elution order, irreversible adsorption, etc.). Therefore, we tried to use the power of the recently introduced multiple mass spectrometric (MS^n) instruments based on ion trap technology (Finnigan MAT LCQ). Throughout this paper the abbreviation MS^n (*e.g.*, MS^3) is preferred to MS-MS-MS, etc., because of its simplicity when n is > 2. MS^n gives structural information on an underivatised compound by sequential fragmentation. No information was found in the literature on the identification of corticosteroids using MS^n.

It was found that an extract of an injection site or of an unknown cocktail could be directly infused into the interface of the mass spectrometer. In this way, all the components of the extract are transferred into the mass spectrometer at the same time. In this paper, a rapid detection method following a TLC screening is described. A method was developed for the identification of a number of target and "unknown" CoST by direct MS^n analysis. Tables that summarise the mass data for

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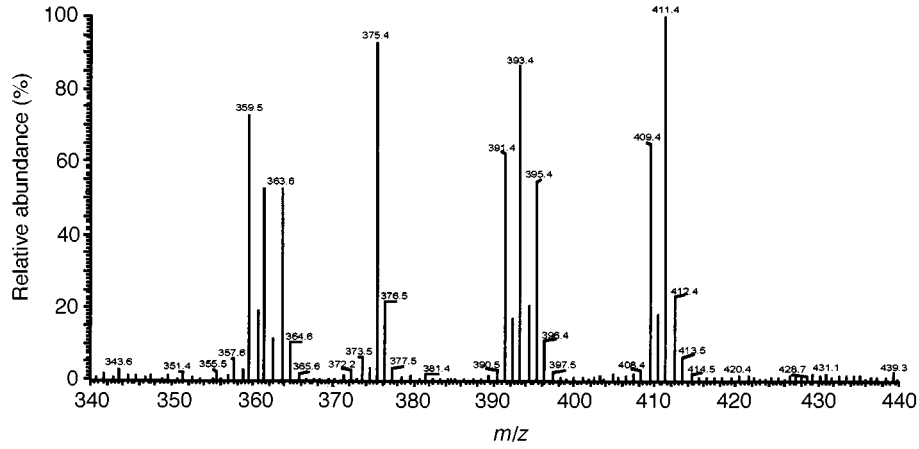

Fig. 1 Full MS^1 spectrum of a mixture of eight CoST.

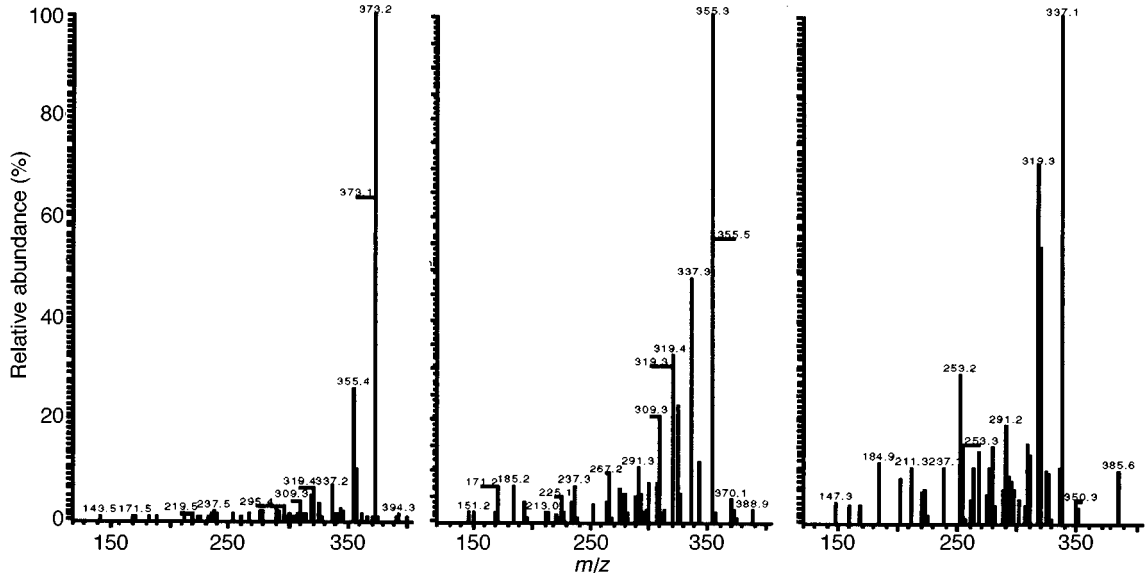

Fig. 2 Full MS^2, MS^3 and MS^4 spectra of dexamethasone.

target and "unknown" CoST are presented. An example of the identification of a "new" corticosteroid in animal fattening, clobetasol propionate, is presented.

2. Experimental

2.1. Apparatus

The following apparatus was used: homogeniser (*e.g.*, Waring blender with 250 ml reservoir, Ultra-Turrax), Stomacher 400 Lab Blender (Seward Medical, London, UK), microwave oven, centrifuge, rotary vacuum evaporator, water-bath, extraction flasks (250 and 500 ml), vacuum manifold (*e.g.*, Sample Preparation Unit, Analytichem International, Harbor City, CA, USA), nitrogen evaporator (*e.g.*, Techni Dry Block) or other types of evaporators (*e.g.*, Speedvac SVC 200, SC 210A, Savant Instruments, Farmingdale, NY, USA; Howe Gyrovap, VA Howe & Co. Ltd., Banbury, Oxon, UK), chromatographic columns and tanks.

The mass spectrometer used was an LCQ Ion Trap Mass Analyzer (Finnigan MAT, San Jose, CA, USA) with an electrospray interface.

Table 1 Diagnostic ions (*m*/*z*) for the identification of target CoST

Analyte	MH^+	Diagnostic ions in MS^2
Btm (betamethasone)	393	373, 355, 337
Dxm (dexamethasone)	393	373, 355, 337
FlM (flumethasone)	411	391, 371, 335
FML (fluorometholone)	377	357, 339, 321
Bcm-DP (beclomethasone dipropionate)	521	503, 411, 319
Clol-P (clobetasolpropionaat)	467	447, 373, 355
Dom (desoximetasone) (internal standard)	377	357, 339, 321

2.2. Reagents and reference components

Most reference CoST were obtained from Steraloids (St) (Wilton, NY, USA) or Sigma (Si) (St-Louis, MO, USA). Other CoST were gifts from various sources. All recent standards were obtained through the Belgian NRL [National Reference Laboratory, WIV-LP (formerly IHE), Brussels, Belgium] to ensure that all the field laboratories used the same standards.[17] The most important CoST and their common abbreviations in Belgium (cited in order of increasing molecular mass) used in this investigation are as follows: prednisone (Pron, St P300) (17,21-dihydroxypregna-1,4-diene-3,11,20-trione); predniso-

Table 2 List 01

CoST Ester	MM	MM +	Molecular mass 358.2 Pron	360.2 Prolon	360.2 Cron	362.2 Crol	374.2 MProlon	376.2 FML	392.2 Dxm	394.2 Trm	408.2 Bcm
Acetonide		40.0	398.2	400.2	400.2	402.3	414.3	416.2	432.2	434.2	448.2
Acetate	60.1	42.0	400.2	402.2	402.2	404.2	416.2	418.2	434.2	436.2	450.2
Propionate	74.1	56.1	414.2	416.3	416.3	418.3	430.3	432.3	448.3	450.2	464.2
Butyrate	88.1	70.1	428.3	430.3	430.3	432.3	444.3	446.3	462.3	464.3	478.3
Diacetate	102.1	84.0	442.2	444.2	444.2	446.3	458.3	460.2	476.2	478.2	492.2
Valerate	102.1	84.1	442.3	444.3	444.3	446.3	458.3	460.3	476.3	478.3	492.3
Pivalate	102.1	84.1	442.3	444.3	444.3	446.3	458.3	460.3	476.3	478.3	492.3
Caproate	116.2	98.1	456.3	458.3	458.3	460.4	472.4	474.3	490.3	492.3	506.3
Benzoate	122.1	104.1	462.3	464.3	464.3	466.3	478.3	480.3	496.3	498.3	512.3
Dipropionate	130.1	112.1	470.3	472.3	472.3	474.3	486.3	488.3	504.3	506.3	520.3
Enanthate	130.2	112.2	470.3	472.4	472.4	474.4	486.4	488.4	504.4	506.3	520.3
Phosphate (di-Na)		124.0	482.1	484.2	484.2	486.2	498.2	500.2	516.2	518.1	532.1
Cypionate	142.2	124.1	482.3	484.3	484.3	486.3	498.3	500.3	516.3	518.3	532.3
Caprylate	144.2	126.2	484.4	486.4	486.4	488.4	500.4	502.4	518.4	520.4	534.4
Phenylpropionate	150.2	132.2	490.3	492.4	492.4	494.4	506.4	508.4	524.4	526.3	540.3
Nonanoate	158.2	140.2	498.4	500.4	500.4	502.4	514.4	516.4	532.4	534.4	548.4
Tosylate	172.2	154.2	512.4	514.4	514.4	516.4	528.4	530.4	546.4	548.4	562.4
Decanoate	172.3	154.2	512.4	514.4	514.4	516.5	528.5	530.4	546.4	548.4	562.4
Divalerate	186.2	168.2	526.4	528.4	528.4	530.4	542.4	544.4	560.4	562.4	576.4
Undecylate	186.3	168.3	526.4	528.4	528.4	530.5	542.5	544.5	560.5	562.4	576.4
Laurate	200.3	182.3	540.5	542.5	542.5	544.5	556.5	558.5	574.5	576.5	590.5

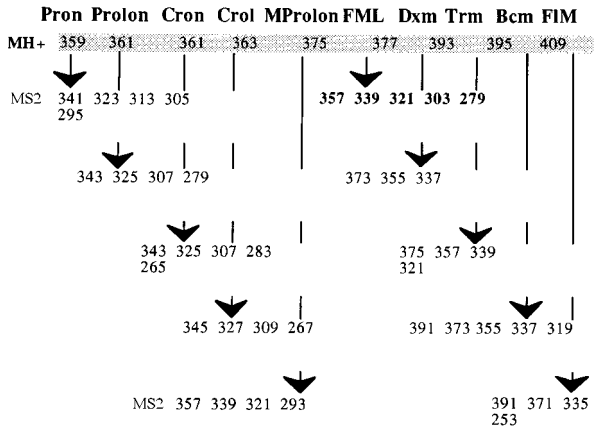

Fig. 3 Overview of the fragmentation of list 01 components.

lone (Prolon, Si P6004) (11β,17,21-trihydroxypregna-1,4-diene-3,20-dione); cortisone (Cron, St Q2500) (17,21-dihydroxypregn-4-ene-3,11,20-trione); cortisol (Crol, St Q3880) (11β,17,21-trihydroxypregn-4-ene-3,20-dione); methylprednisolone (MProlon, Si M0639) (11β,17,21-Trihydroxy-6a-methylpregna-1,4-diene-3,20-dione); fluorometholone (FML, F9381) (9-fluoro-11β,17-dihydroxy-6a-methylpregna-1,4-diene-3,20-dione) ; dexamethasone (Dxm; Si D1756) (9-fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione); betamethasone (Btm, Si B7005) (9-fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione); triamcinolone (Trm, Si T6376) (9-fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione); beclomethasone (Bcm, Si B0385) (9-chloro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione); flumethasone (Flm, Si F9507) (6α,9-difluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione); clobetasol (Clol) (21-chloro-9-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione); and the propionate esters of Btm, Bcm and Clol. Clol itself is not commercially available.

The internal standard was desoximetasone (Dom, Si D6038) (9-fluoro-11β,21-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione).

All solvents were of analytical-reagent grade from Merck (Darmstadt, Germany).

2.3. Solutions

Stock standard solutions of CoST were prepared at 200 ng μl^{-1} in ethanol. Tenfold dilutions of these solutions gave working standard solutions of CoST at a concentration of 20 ng μl^{-1}.

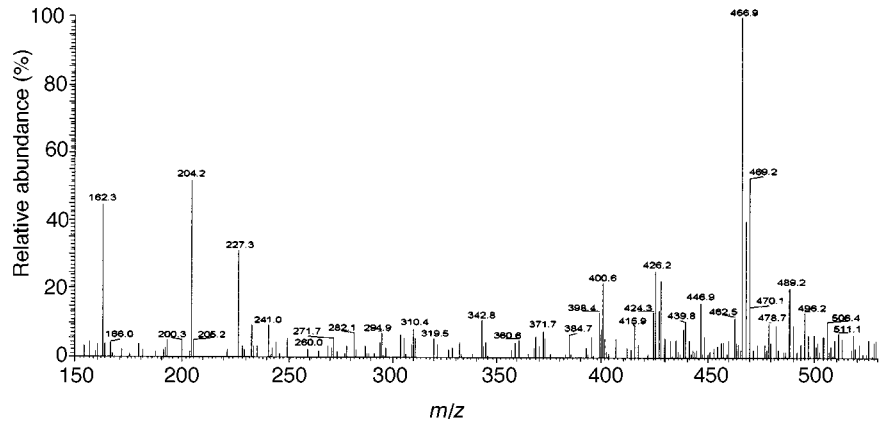

Fig. 4 MS^1 spectrum during infusion of injection site with unknown component.

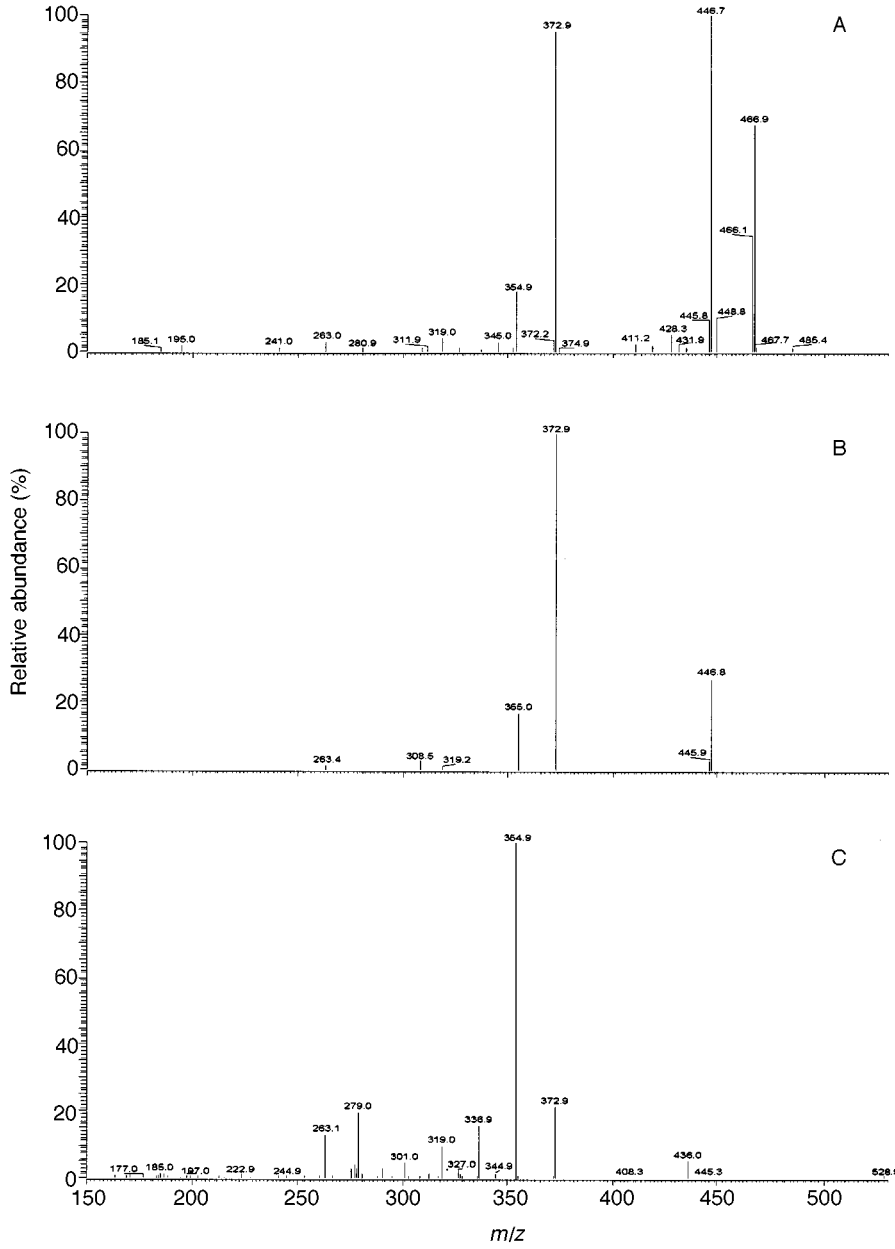

Fig. 5 A, MS^2, B, MS^3 and C, MS^4 spectra of the unknown component in the injection site.

2.4. HPTLC screening of corticosteroids

The injection site is sampled by cutting at least 2 g suspect material with a bistoury and transferring it into a double bag of a stomacher. The number of the sample is marked on the outer bag. Methanol is added at a ratio of 2.5 ml g^{-1} material (with a minimum of 5 ml). Internal standard desoximetasone (Dom) (15 μl = 3000 ng per 2.5 ml of methanol or 3 μg kg^{-1}) is added. For the destruction of the matrix, a stomacher is used for at least 1 min. Overnight extraction allows the matrix to react longer with the extraction solvent and therefore a higher extraction yield is obtained. After extraction overnight, the mixture is filtered into a disposable plastic recipient. This primary extract is prepared in a laboratory room separated from the laboratory for residue analysis to avoid contamination problems. A primary extract of a suspect cocktail is prepared by mixing or extraction with methanol in an analogous way.

If necessary, an aliquot (90%) of this extract may be concentrated: 4.5 ml of extract are evaporated to dryness and the residue is dissolved in 0.8 ml of methanol. Subsequently 1.2 ml of water is added and the mixture is placed in an ultrasonic bath until the solution is clear. A C_{18} cartridge (3 ml, 500 mg) is conditioned with 2 × 2 ml of methanol followed by 2 × 2 ml of water. The extract is transferred into the cartridge and the CoST are eluted with methanol–water (70 + 30 v/v) (2 ml) followed by methanol (2 ml). This eluate is evaporated to dryness and the residue is dissolved in 50 μl of ethanol.

The concentrated extracts (10 μl) are spotted on an NH_2-F_{254} plate together with standard mixtures of the target CoST (600 ng). The plate is developed with ethanol–chloroform–ethyl acetate (10 + 20 + 20 v/v). If this plate shows a response for esters (at the front), another plate is developed with hexane–acetone (65 + 35 v/v).

After drying the plates, compounds are revealed by heating the plates at 200 ± 10 °C for 3 min. The plates are inspected under UV and visible light. At 254 nm the CoST are visible as blue–purple spots on a white background. At 366 nm the spots are beige or blue on a blue–purple background. If suspect spots are present, the extract is transferred for MS^n analysis.

Other procedures for the TLC analysis of CoST have been described earlier[8,9,17] but their compatibility with MS^n was not tested.

Table 3 Part of list 02: some corticosteroid esters matching the MH^+ of the unknown (2361, clobetasol; 2368, clocortolone; 3126, diflorasone)

			Molecular mass		
Ester	MM	MM+	410.17 2361	410.17 2368	410.19 3126
Acetonide		40.04	450.21	450.21	450.23
Acetate	60.05	42.03	452.20	452.20	452.22
Propionate	74.08	56.06	466.23	466.23	466.25
Isobutyrate	88.10	70.08	480.25	480.25	480.27
Butyrate	88.10	70.08	480.25	480.25	480.27
Diacetate	102.06	84.04	494.21	494.21	494.23
Valerate	102.13	84.11	494.28	494.28	494.30
Pivalate	102.13	84.11	494.28	494.28	494.30
Caproate	116.16	98.14	508.31	508.31	508.33
Benzoate	122.12	104.10	514.27	514.27	514.29
Dipropionate	130.12	112.10	522.27	522.27	522.29
Enanthate	130.18	112.16	522.33	522.33	522.35
Phosphate (di-Na)		123.96	534.13	534.13	534.15
Cypionate	142.15	124.13	534.30	534.30	534.32
Caprylate	144.21	126.19	536.36	536.36	536.38
Phenylpropionate	150.18	132.16	542.33	542.33	542.35
Nonanoate	158.23	140.21	550.38	550.38	550.40
Tosylate	172.20	154.18	564.35	564.35	564.37
Decanoate	172.26	154.24	564.41	564.41	564.43
Divalerate	186.22	168.20	578.37	578.37	578.39
Undecylate	186.27	168.25	578.42	578.42	578.44
Laurate	200.31	182.29	592.46	592.46	592.48

2.5. MS^n apparatus and conditions

For MS^n experiments, an LCQ ion trap mass spectrometer was used with a built-in syringe pump. The analytes were ionised through the electrospray interface producing MH^+ or $M-H^-$ ions.

Infusion. The remainder of the concentrated extract used for HPTLC was directly infused into the mass spectrometer. Infusion into the mass spectrometer was performed as follows: the incoming flow of sample (5 μl min^{-1}) was mixed with an eluent flow of, methanol–1% acetic acid (60 + 40 v/v) at 0.3 ml min^{-1} through a T-piece. In order to exclude contamination, the spray shield, heated capillary and infusion line were thoroughly cleaned with methanol before starting the infusion and acquisition, and between samples. Eluent was infused into the mass spectrometer through the infusion line and checked for the presence of known parent (MH^+) and daughter ions, to ensure that no contamination or residues of previous standards or positive samples were present. If this check proved to be negative, a new sample was infused.

Tuning. In theory, a tune file can be made for each compound individually. Since during one acquisition many different compounds are investigated, and since in practice there is only a slight difference in parameter settings to obtain optimum conditions for compounds with a small molecular mass difference and a similar structure, Dxm was used for tuning purposes. An amount (40 ng μl^{-1}) which produces a fairly readily distinguished pseudo-molecular ion (MH^+) was directly infused into the mass spectrometer and the different MS parameters (capillary voltage, tube lens offset, ESI voltage, *etc.*) were optimised and saved in a tune file. This tune file was used during the subsequent investigation.

Interpretation. During infusion, the spectrum was searched for MH^+ ions in the range 350–510 mu which rise above the normal background originating from the infusion liquid and the electronics. For first line quality control, the MH^+ ion of Dom (377u) should be present, otherwise sampling and the extraction procedure should be repeated.

A "suspect" ion can be defined as an ion that rises above the background noise and that needs further fragmentation to match the spectrum with a standard mass spectrum. If a suspect ion does not match any known standard compound, it becomes an unknown. If such an ion was observed the MS^2 and MS^3 spectra of the suspect peak were acquired. For the target components the diagnostic ions are given in Table 1. The sample is considered to be positive when the MS^2 spectrum matches that of the previously infused standard. If, next to or instead of target component ions, other ions are observed, an attempt at the identification of these components is made using the so-called list 01 and 02 (see Results and discussion).

3. Results and discussion

3.1. MS^n of corticosteroid standards

Mixtures of standards of CoST are infused into the mass spectrometer. In Fig. 1 a full MS^1 spectrum of a mixture of eight CoST is shown.

Very abundant MH^+ ions for all CoST infused are found. This is normal because electrospray is a soft ionisation technique. The relative energy of collision applied to pseudo-molecular ions is chosen so the intensity of the most intense daughter ion is maximum. Further fragmentation is performed on the MH^+ for MS^2 and for MS^n, as a general rule, on the most abundant daughter ion. During one acquisition, MS^1 up to MS^3 or MS^4 spectra are acquired. In Fig. 2, as an example, the MS^2, MS^3 and MS^4 spectra for dexamethasone are given. In Fig. 3 an overview of the fragmentation of the most important CoST (so-called list 01 components; see below) is given.

The relative values of the collision energy are comparable for related compounds. The collision energy is reported on a relative scale (percentage) and no correlation is given with an absolute voltage. As the percentage is increased, more fragment ions will be formed. For this application we preferred to apply energy to the MH^+ ion until it disappeared and only daughter ions were present. For further fragmentation of the most intense daughter ions, the same rule was applied. A collision energy of about 20% will be sufficient to generate a good response of fragment ions.

3.2. MS^n of corticosteroids in injection sites

It was found that the extract of an injection site (or an unknown cocktail) could be directly infused into the interface of the mass spectrometer. When a fairly high concentration of analyte(s) is present (which is mostly the case in a "positive" injection site), the MH^+ ions will rise above the background ions. If the concentration is lower, the pseudo-molecular ions will disappear into the background.

For the identification of CoST, the following three stage strategy is used. In addition to the target components (stage 1) a so called "list 01" is programmed in Microsoft Excel (stage 2). In this list 12 important "known" CoST are listed in columns from left to right in order of increasing molecular mass. In this list 01, Dxm, Btm and Pam (paramethasone) are in the same column and thus indistinguishable. In the rows, 21 acids (possibly used for esterification of CoST) are listed also in order of increasing molecular mass. The combination of columns and rows yields 212 possible esters of the CoST. For the mass of the different esters the loss of water (−18) upon formation of the ester is taken into account. In Table 2 the list 01 is given. The

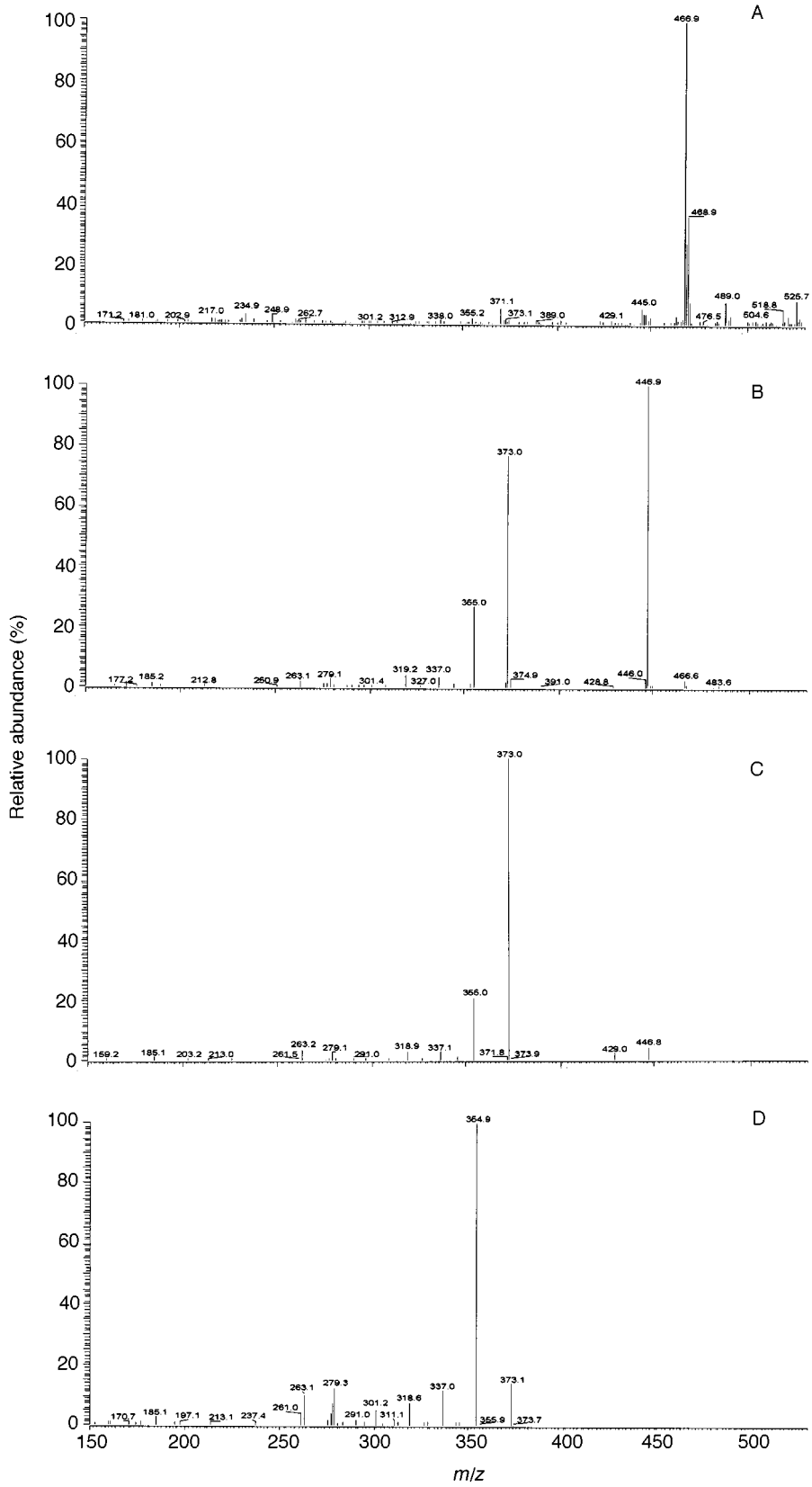

Fig. 6 A, MS^1, B, MS^2, C, MS^3 and D, MS^4 spectra of clobetasol propionate.

masses of the esters increase from left to right and from top to bottom, making the search for a certain mass easy.

If, in an MS^1 spectrum of a sample an abundant "non-target" MH^+ ion is observed, list 01 is searched for a match with the corresponding molecular mass (stage 2). If a match is found, a search is made to establish if the component is available in one of our laboratories. If so, MS^n spectra of both sample and standard are acquired and compared.

3.3. Validation of the procedure

In the list 01, only Dxm, Btm, Flm, Fml, Bcm-DP and Clol-P (see section 3.5) are validated because they are the target components in Belgium. Validation was carried out by fortifying blank tissue samples resembling the structure of an injection site with known amounts of CoST at the level of 2 ppm and carrying out the procedure described several times (> 20).

The number of times that the CoST added are detected is statistically evaluated. To our clients (in this case the inspection services) it can be guaranteed that target CoST present in injection sites at the level of 2 ppm will be detected by the laboratory with a probability (frequency) of > 95%.

3.4. MS^n identification of a "new" corticosteroid

In an injection site, a suspect HPTLC spot at the correct R_F value of beclomethasone dipropionate (Bcm-DP) and/or betamethasone dipropionate (Btm-DP) was observed (one-dimensional HPTLC). Using co-chromatography a new, not completely separated, TLC spot occurs. The suspect spot also has a slightly different colour than the Btm and Bcm esters. According to the quality criteria described in EC 93/256,[18,19] the sample was considered to be negative (= analyte absent or lower than the action limit). Further investigations with MS^n were carried out.

The extract of the injection site was infused in the LCQ, next to Bcm-DP and Btm-DP. The spectrum of the unknown showed an intense ion at *m*/*z* 466.9 (most probably MH^+) (Fig. 4). This ion is clearly different from the MH^+ ion observed for the standards of Bcm-DP and Btm-DP (*m*/*z* 521.8 and 505.7 respectively; see list 01) and is also different from the target components. In list 01 only one match with cortisol benzoate was found. However, this molecule does not correspond with the other characteristics of the MS^1 spectrum.

For the identification of this "unknown", the following points were taken into account: the spot migrates a long distance and has a similar R_F value to esters of CoST. A CoST ester is a possibility. The spot is also present in the 2D-HPTLC of anabolics. The presence of two isotope peaks with an *m*/*z* difference of 2 and a ratio of 3:1 indicate that the analyte contains one chlorine atom. The loss of 20 u in MS^2 indicates a loss of HF. This corresponds with the findings for other CoST standards containing fluorine (Fig 3). The loss of 74 u indicated the presence of propionic acid. Both Bcm-DP and Btm-DP lose the 74 u fragment twice. The fragmention of the molecule is shown in Fig. 5. One of our laboratories has observed this spot also more than once in illegal cocktails in addition to other target components.

In Microsoft Excel, a combination of all CoST in the Merck Index (except those in list 01) with all the acids already used in list 01 was made (stage 3). The CoST are indicated by their Merck Index number and listed in order of increasing molecular mass. This so-called list 02 contains 20 CoST combined with 22 esters, yielding 440 possible CoST esters. In this list 02, three matches with the molecular mass of the unknown are found (Table 3): the monopropionate esters of 2361 (clobetasol), 2368 (clocortolone) and 3126 (diflorasone). Diflorasone is eliminated because the molecule does not contain chlorine.

In the Sigma catalogue, one of these products was found and ordered: clobetasol propionate. A solution of the standard was infused and it was found that the MS^n spectra matched the spectra of the unknown (Fig. 6). Only in the MS^1 spectrum were substantial differences in the low mass region observed (Fig. 4). These were most probably formed by co-extracted components from the matrix.

Based on the so-called "intellectual owner's right" (a Belgian tradition which states that the discoverer of a "new" component may propose an abbreviation), the abbreviation ClolP was proposed for clobetasol propionate. The samples and the analytical data were transferred to the Belgian NRL and the Community Reference Laboratory (CRL), the RIVM at Bilthoven. The formula of clobetasol propionate and possible fragmentations is shown in Fig. 7.

The base peak in the MS^2 spectrum (*m*/*z* 447) is formed by the loss of HF (−20 u) (like all other fluorine-containing CoST). In the MS^3 spectrum the loss of propionic acid (−74 u) is predominant (base peak at *m*/*z* 373). The MS^4 spectrum shows several losses of water (−18 u) with the formation of ions at *m*/*z* 355, 337, 319 and 301. By increasing the isolation width of the parent ion and the most intense daughter ions, ions containing the chlorine isotopes are also isolated. During further fragmentation the isotope peaks remain present in the spectrum. This means that chlorine is not split off.

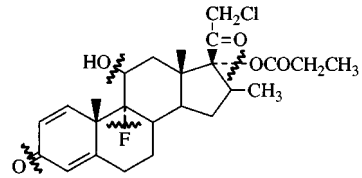

Fig. 7 Clobetasol propionate and possible fragmentation.

4. Conclusion

A possible strategy for the control of the abuse of CoST in cattle fattening through the analysis of injection sites and illegal cocktails has been described. The presence or absence of CoST is screened by HPTLC, which is a very fast and robust analytical technique. The results for negative samples may be produced very quickly (*e.g.*, within 2 d).

If, on the TLC trace, suspect spots are present, the extract is subjected to the powerful three stage MS^n identification procedure. This confirmation step by direct infusion is also very fast because no chromatographic run is needed. Moreover, with this technique not only the presence of target (stage 1) or "known" (stage 2) CoST may be confirmed, but also "unknown" components (stage 3) may be identified. An example of such an identification is that of clobetasol propionate.

However, this technique also has its limitations: the differentiation power between isomers such as betamethasone and dexamethasone is low. For such differentiation, other techniques have to be used. Also, true unknowns, not belonging to any group of analytes with which our laboratory is used to dealing, can be present in the sample. If this unknown is seen fairly often when performing routine screening tests and our confirmation technique is unable to make a valid identification, other techniques such as NMR spectroscopy are advisable options.

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