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High-throughput analysis of growth promoters by LC-MS/MS using a novel tube plasma ionisation source

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ABSTRACT

Background: The use of estrogenic compounds to promote growth in livestock animals is prohibited in the European Union. They are typically determined by gas chromatography coupled to mass spectrometry (GC–MS) using the electron ionisation source (EI). However, the high fragmentation observed by EI requires large injection volumes to reach the analytical requirements. Also, the need for time-consuming derivatisation makes this approach not desired for routine analysis. Besides, liquid chromatography-mass spectrometry (LC-MS) methodologies using electrospray ionisation (ESI) shows also inefficient ionisation and high matrix effects and thereby, reliable alternatives are needed to improve the throughput of the analysis of estrogenic compounds.
Results: In this work, a recently described ionisation source called tube plasma ionisation (TPI) is used to overcome the limitations of LC-MS determination of growth promoters, as it enhances the range of ionisable compounds, covering both polar and non-polar substances. After careful evaluation of source and MS related parameters and optimization of an HPLC separation based on a water/methanol gradient programme, a comparison was made to a commercial ESI source. TPI showed lower detection limits instrumentally (0.007 to 5.1 μg L^{-1} compared to 0.112 to 394 μg L^{-1} with ESI) but also in both bovine urine and meat matrices. Additionally, the sensitivity of the determination is increased when using TPI (median: 0.66) over ESI (median: 0.20). The novel approach shows high linearity, precision (both intra- and inter-day) and trueness and significantly increases the analytical workflow compared to ESI, allowing a proper quantification of the compounds in complex matrices.
Significance: The relevance of this works lies on the capabilities of the LC-MS system using TPI to outperform ESI, ensuring the monitoring of growth promoters in livestock animal matrices at ultra trace levels. This new source not only guarantees a proper analytical performance, but it also helps to simplify time-consuming and tedious sample treatment steps such as derivatisation for low-polar compounds.

1. Introduction

The use of growth-promoting substances in animals to enhance the fattening process in livestock production and to improve animal performance in competitions has been extensively described for decades [1]. However, these practices have a significant impact on both animal welfare and human health [2,3], as products of animal origin, such as meat or milk, may contain pharmaceutical residues [4]. Under this scenario, the European Union (EU) banned certain substances having a hormonal or thyrostatic action, including estrogenic compounds, in

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livestock farming with Council Directive 96/22/EC [5] to prevent adverse effects of veterinary products on consumers. Additionally, under the Official Control Regulation (EU) 2017/625/EC [6], the EU prohibit the use of hormones to promote growth, thereby establishing the legal framework for controlling residues in livestock production. Otherwise, in the United States and several other countries, growth promoters are still used in medicated animal feed [7], which could, therefore, be present in imported animal-derived products.

In national residue control monitoring plans, these anabolic compounds are analysed in various biological matrices, including meat and urine. To identify and quantify banned hormones in these matrices, it is necessary to use highly sensitive methods, using mass spectrometry (MS) as a confirmatory technique [8]. Therefore, the methods that are routinely used are based on MS. [9] In routine analysis, in general, the technique of choice for the determination of estrogenic compounds is gas chromatography (GC) coupled to MS, either by tandem mass spectrometry (MS/MS) [10] or high-resolution mass spectrometry (HRMS) [9]. The use of electron ionisation (EI) by GC–MS results in high fragmentation of target compounds [11] and, therefore, requires large injection volumes to reach the general concentration ranges of the target compounds (0.1–2 ng on column). Moreover, the requirement of a derivatisation step to improve their volatility and thermal stability leads to time-consuming sample preparation, which is less practical for routine control monitoring laboratories. To overcome these complexities by GC–MS, owing to the improvements on liquid chromatography (LC) technology and increased versatility of this analytical platform, LC-MS has been evaluated on several occasions [12]. However, the aromatic ring structure and lack of keto groups in estrogenic steroids make their detection by LC–MS more complicated since it is hampered due to their low ionisation efficiency by commonly used electrospray ionisation (ESI) [13,14]. To overcome this, different chemical derivatisation can be used by introducing permanently charged or easily protonated moieties, despite making sample preparation and clean-up more complex [13–15]. For this reason, reliable alternatives are needed to improve the throughput of the analysis of estrogenic compounds.

In LC-MS approaches, ionisation is most commonly achieved using ESI [14,16–18]. Despite its suitability for polar compounds, its performance with mid- and low-polar compounds is quite limited, requiring alternative ion sources such as atmospheric pressure chemical ionisation (APCI) and photoionisation (APPI) [19–22]. Recently, the use of cutting-edge atmospheric pressure plasma-based ionisation sources is increasing in both GC–MS and LC-MS approaches due to their high capacity to ionise polar and non-polar compounds, extending the chemical space of analytical methodologies [23,24]. In these sources, plasma is ignited from a discharge gas, most commonly Argon or Helium. Among them, plasma ionisation based on dielectric barrier discharge (DBD) is gaining interest as it presents a simple experimental set-up and comparably low power consumption. DBD ionisation (DBDI) was introduced in 2007 by Na et al. [25,26] when they applied an alternating current high-voltage to generate a low-temperature plasma that provided soft ionisation of analytes [25–27]. A year later, Harper et al. [28] presented the low temperature plasma (LTP) source, where a glass tube dielectric is used between an outer ring electrode and an internal grounded electrode, instead of a glass plate for sample introduction as with DBDI [29], increasing simplicity in building and operating the ion source and reducing the power consumption [30]. Based on the LTP set-up several changes have been reported, with the most prominent being inverse voltage LTP (iLTP) and flexible micro tube plasma source (FμTP) [27,31,32]. With major advantages being lower required voltages to ingite the plasma and higher ion efficiencies [32]. Recently, another iLTP-based source, so-called tube plasma ionisation (TPI), was developed for GC–MS coupling [33]. The major difference of FμTP and TPI from previous DBDI sources is the absence of the grounded electrode, which further simplifies the source configuration while maintaining proper analytical performance, thereby broadening the range of ionisable substances compared to other atmospheric pressure ionisation (API) sources [33–35]. Further, the elimination of the ground electrode also increases the ease of miniaturization of the source [32]. In addition to the increased simplicity, improved sensitivity was proven for TPI over iLTP [36].

In this study, the potential of the novel plasma-based TPI source for LC-MS coupling is assessed to achieve sensitive and selective quantification of estrogenic compounds in biological samples. A LC-TPI-MS/MS method has been developed, and the most critical working parameters, such as plasma-related parameters (i.e., discharge gas, voltage, frequency, etc.) and source-related parameters (temperatures, flows, and voltages) were thoroughly evaluated and discussed. Additionally, the mobile phase has been optimised to ensure both chromatographic separation and optimal ionisation efficiency. After that, the figures of merit using both standards and biological matrices of the developed LC-TPI-MS/MS methodology have been compared with those from LC-ESI-MS/MS.

2. Materials and methods

2.1. Chemicals and reagents

Thirty-eight commercially available growth promotor analytical standards and 14 labelled standards were obtained from different suppliers. 17β-1-testosterone (17β-1-T), 17β-estradiol (β-E2), 17β-nortestosterone (β-NT), 17β-testosterone (β-T), 17α-testosterone (α-T), 17α-nortestosterone (α-NT), methyltestosterone (MT), 5β-estrane-17β-ol-3-one (β-EEO), 5α-estrane-17β-ol-3-one (α-EEO), dienestrol (DE), mestranol (MEST), methenolone (METH), methandriol (MAD), norgestrel (NORG), normethandolone (NMD), norandrostendiol (NAR), norandrostandion (NATAD), norandrostendion (NATD), norandrosteron (NA), noretiocholanolone (NE) and norepiandrosteron (NEDT) were purchased from Steraloids (New Port, Rhode Island, USA). Estrone (E1), ethynylestadiol (EE2), hexestrol (HEX), progesterone (P), diethylstilbestrol (DES), methylboldenone (MB), stanozolol (DHT) and 4-chloro-4-androst-3,17-dione (CLAD) were purchased from Sigma-Aldrich (San Luis, Misuri, USA). 17α-1-testosterone (17α-1-T) was obtained from BDG synthesis (Wellington, New Zealand), 17α-estradiol (α-E2) was purchased from Carbosynth (now Biosynth, Compton, United Kingdom), 17α-ethyl-5β-estrane-3α,17β-diol (EED) and 16β-OH-stanozolol (β-SOH) were provided by the National Measurement Institute (Department of Industry, Science and Resources, Canberra, Australia), 17α-methyl-5β-androstan-3α,17β-diol (MEAD I) was obtained from Cerilliant (Round Rock, TX, United States), benzestrol (BENZ) was purchased from BOC sciences (Shirley, NY, United States), 17β-chlorotestosterone (β-CT) was obtained from Cayman Chemical (Ann Arbor, MI, United States) and norethandrolone (NED) was purchased from LGC (Teddington, United Kingdom). 17α-chlorotestosterone (α-CT), diethylstilbestrol-d6 (DES-d6), 17β-testosterone-d3 (T-d3), 17β-nortestosterone-d3 (NT-d3), 17β-estradiol-d3 (E2-d3), estrone-d3 (E1-d3), methyltestosterone-d3 (MT-d3), methylboldenone-d3 (MB-d3), ethynylestradiol-d4 (EE2-d4), progesterone-3-13C (3-13C-P), chlorotestosterone-d3 (CT-d3), dienestrol-d4 (DE-d4), hexestrol-d4 (HEX-d4), noretiocholanolone -d4 (NE-d4), Norandrosteron-d4 (NA-d4) are provided by European Union Reference Laboratory for growth promoters (Wageningen, The Netherlands). Individual stock standard solutions were prepared in methanol at a concentration of 1000 mg L^{-1}. An intermediate standard mixture containing all the target compounds (10 mg L^{-1}) was prepared from standard stock solutions by appropriate dilution in methanol. Working standard solutions were prepared by corresponding dilution from the intermediate standard mixture solution. All these standard solutions were stored at −20 °C until their use.

Ultrapure water was obtained at a resistance of 18.2 MΩ cm using a Milli-Q-system (Millipore, Billerica, MS, United States) or an Atrium Pro VF ultrapure water system (Sartorius Stedim Biotech, Göttingen, Germany). Methanol (≥ 99.9 %), used as solvent, was obtained from Actu-All Chemicals (Oss, The Netherlands) in ultra-LC-MS grade or from VWR

Chemicals (Leuven, Belgium) in HPLC/LC–MS grade. Acetonitrile and isopropanol (≥ 99.9 %) were both obtained from Sigma Aldrich (Darmstadt, Germany) in LC-MS grade. Acetic acid, used as a mobile phase modifier, was purchased from Sigma-Aldrich (Darmstadt, Germany) in LC–MS grade (100 % purity) and acetic acid, used for sample preparation, was obtained from Merck (Darmstadt, Germany). Formic acid was purchased from Fisher Chemicals (Geel, Belgium) in LC–MS grade. Ammonium acetate (ACS reagent grade), ammonium formiate (≥ 99.0 %), sodium acetate (ACS reagent grade), and β-Glucuronidase/arylsulfatase (MQ100 quality) from *Helix pomatia* were purchased from Merck (Darmstadt, Germany). Tert-butyl-methyl-ether (TBME), n-heptane and n-pentane were purchased from Biossolve (The Netherlands), whereas tris(hydroxymethyl)-aminomethane (TRIS) from Sigma-Aldrich (San Luis, Missouri, USA). n-hexane was obtained in GC–MS grade from Supelco (Merck, Darmstadt, Germany). SPE Bond Elute C18 (500 mg, 6 mL) cartridges were purchased from Agilent Technologies (Santa Clara, California, USA). Argon, used as plasma gas, was obtained from Air Liquide (Düsseldorf, Germany) with 99.998 % purity. Helium was purchased in 99.999 % purity from Air Liquide (Düsseldorf, Germany) and nitrogen, used as drying gas, was obtained from Air Liquide (Düsseldorf, Germany) with 99.999 % purity.

2.2. Samples and sample treatment

Samples were from Wageningen Food Safety Research (WFSR) in-house stock and the presence of estrogenic compounds had been checked previously by GC–MS/MS. In total, 2 bovine muscles and 2 bovine urine samples were provided for this study.

2.2.1. Muscle samples

One gram of muscle was spiked with 30 µL of a 0.1 mg L^{-1} internal standard solution. Thereafter, 10 mL of water was added, and the sample/water mixture was first vortexed for 30 s and then ultrasonicated for 15 min. To the ultrasonicated mixture, 10 mL of TBME was added, and the sample was rotated for 15 min. Later, for 15 min, a centrifugation step at 4500*g* was performed, and the sample tubes were placed in an ultra-freezer for 20 min. Tubes were put through a decantation step, and the TBME layer was extracted into a 10 mL tube for evaporation under a gentle stream of nitrogen at 55 °C. The TBME extraction was repeated and both evaporated extracts were combined and dried until dryness. The extract was reconstituted in 4 mL methanol/water (80:20, *v/v*) mixture and vortexed and ultrasonicated for 30 and 60 s, respectively. To continue, 4 mL of *n*-heptane was added, and centrifugation was carried out at 2200*g* for 10 min. The *n*-heptane layer was then discarded. This last washing step was repeated once, and both extracts were evaporated until the volume was below 0.5 mL. Before carrying out the purification by SPE C18 (*section 2.2.3*), water was added until 4 mL.

2.2.2. Urine samples

Five millilitres of urine were spiked with 30 µL of a 0.1 mg L^{-1} internal standard solution. Thereafter, 1 mL of 2.0 M acetate buffer was added, and after vortexing, the pH was checked to make sure it was within the range of 5.2 ± 0.2. Enzymatic hydrolysis was carried out by incubation at 50 °C for two hours with 25 µL of β-Glucuronidase/arylsulfatase from *Helix Pomatia* (1/10 diluted with acetate buffer, *v/v*). After cooling the samples to room temperature, 10 mL of TBME was added, and the mixture was vortexed for 30 s. Later, for 10 min, a centrifugation step at 4500*g* was performed, and the sample tubes were placed in an ultra-freezer for 20 min. Tubes were put through a decantation step, and the TBME layer was extracted into a 10 mL tube for evaporation under a gentle stream of nitrogen at 55 °C. The TBME extraction was repeated, and both evaporated extracts were combined and dried until dryness. Before carrying out the purification by SPE C18 (section 2.2.3), the extracts were dissolved in 0.2 mL of methanol, and water was added to a final volume of 4 mL.

2.2.3. Extract purification with SPE C18

This step was carried out with the obtained extracts for muscle *(section 2.2.1)* and urine *(section 2.2.2)*. A Bond Elute C18 (500 mg, 3 ml) cartridge was conditioned with 5 mL methanol followed by 5 mL of water. After loading the sample, the cartridge was washed using 5 mL of water and 5 mL of methanol/water (40:60, *v/v*). The analytes were eluted from C18 cartridges with 5 mL of methanol/water (80:20, *v/v*). The eluate was evaporated until <0.5 mL under a stream of nitrogen at a temperature of 55 °C. The residue was dissolved in 2.5 mL of tris buffer and extracted with 6 mL of *n*-pentane. Centrifugation was carried out for 10 min at 2200 *g*, and the tubes were placed for 20 min in an ultra-freezer. Thereafter, the *n*-pentane layer was transferred into a clean tube and evaporated at 40 °C under a gentle stream of nitrogen. The *n*-pentane extraction was repeated, and the combined extract was evaporated until dryness. Extracts were reconstituted in 50 µL of ethanol and transferred into injection vials.

2.3. LC − MS instrumentation

For the chromatographic separation of the growth promoters a 1290 Infinity II HPLC (Agilent Technologies, Santa Clara, CA, United States) was used with a Kinetex 17u C18 100 A column (100 × 2.1 mm, 1.7 µm, Phenomenex, Torrance, CA, United States). As mobile phase components, water with 0.1 % (*v/v*) acetic acid (solvent A) and methanol with 0.1 % (*v/v*) acetic acid (solvent B) were used. The elution gradient was as follows: initial conditions, a 40 % B isocratic step (held 2 min) and raised to 50 % B in 0.5 min (held 13.5 min), from 50 % to 80 % B in 9 min and from 80 % to 100 % B in 1 min (held 3 min) before returning to the initial conditions (3 min of equilibration time). During the whole analysis (31 min) the flowrate was set at 0.3 mL min^{-1}. Standards were injected from 300 µL fixed insert vials (Thermo Scientific, Langerwehe, Germany) with a 1290 Multisampler (Agilent Technologies, Santa Clara, CA, United States) using 5 µL as injection volume.

The LC was coupled to a 6470 LC/TQ triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, United States) equipped with both ESI and a lab-made TPI source (Fig. 1).

The TPI probe was built in-house at the Applied Analytical Chemistry

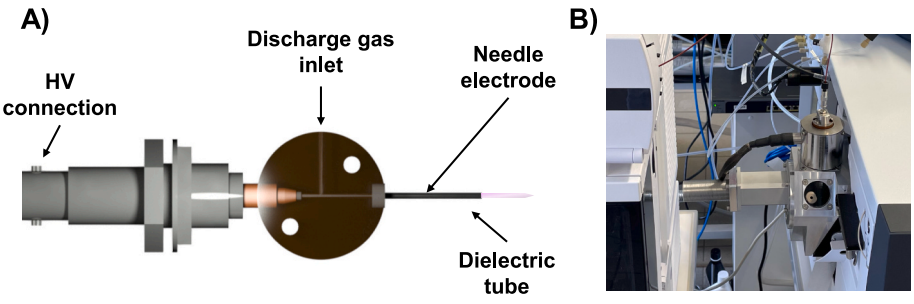

Fig. 1. A) Scheme of the TPI probe and B) housing for the LC-configuration.

group of the University of Duisburg-Essen [33]. In the TPI source, a needle electrode is placed inside a quartz tube with an inner diameter of 0.6 mm. The needle electrode is connected to the high voltage supply and is positioned opposite to the MS inlet. The LC eluate is delivered from the top of the source at a 90° angle into the source. The high voltage is provided by a PNC 6000–100 ump (Heinzinger, Rosenheim, Germany) and connected to a GHTS 60 A fast high voltage switch (Behlke Power Electronics, Kronberg i. Taunus, Germany). High voltage pulses are generated by a DSO-X 2022 A digital storage oscilloscope (Agilent Technologies, Santa Clara, CA, United States). The TPI plasma was operated at a frequency of 7.5 kHz with a high voltage of 2.0 kV. The pulse width was set to 4.5 µs, and the plasma gas was introduced with 325 mL min^{-1}. The following source conditions were selected after optimisation: gas temperature of 350 °C with a gas flow of 7 L min^{-1}. The vaporiser temperature was set to 500 °C, and the nebuliser pressure to 60 psi while capillary voltage was set to 1000 V. For comparison studies with ESI, an Agilent Technologies (Santa Clara, CA, United States) ESI source was used. After optimisation, the gas temperature in the source was set to 250 °C with a gas flow of 13 L min^{-1}. The nebuliser was used at a pressure of 20 psi. Capillary voltage was set to 6 kV and nozzle voltage to 500 V. The sheath gas was introduced with 3 L min^{-1} at 150 °C. For quantitative analysis, multiple reaction monitoring (MRM) in positive ion mode was used in both ion sources. Instrument control was done using MassHunter Data Acquisition software (Agilent Technologies, Santa Clara, CA, United States). For data evaluation, MassHunter Qualitative Analysis 10.0 (Agilent Technologies, Santa Clara, CA, United States) and Skyline-daily (University of Washington, WA, United States) were used.

2.4. Quality control and method performance characteristics

For quality control, regular QC samples containing a reduced mix of analytes and blanks were run on the analytical system to monitor performance changes and contamination in the system. Working surfaces were cleaned with isopropanol. Cleaning of the HPLC and MS instruments was done according to the manufacturers' standard operating procedures. The ion source was cleaned depending on demand with isopropanol. More resistant deposits on the surface of the MS were typically seen after injections with matrix and were cleaned by ultrasonication of contaminated parts in water with 0.1 % (*v/v*) formic acid, followed by ultrasonication in methanol and finally ultrasonication in *n*-hexane.

The methods were validated according to AQC-Guidelines (SANTE/11312/2021) [37]. The matrix effect (ME, %) was estimated as the relative difference between the peak area observed in the analysis of the spiked blank extract and that obtained from growth promoter's standard at the same concentration level. A negative value in the matrix effect reflects a matrix suppression whereas a positive value means a matrix enhancement in the response. Additionally, the matrix effect was considered negiglible within ±20 % and acceptable within ±20–40 %. Instrumental and method limits of detection (iLODs and mLODs) and limits of quantification (iLOQs and mLOQs) were estimated as S/N of 3 and 10, respectively. Moreover, linearity within the working concentration range (1–750 µg L^{-1} for standard solutions and 0.4–300 ng kg^{-1}

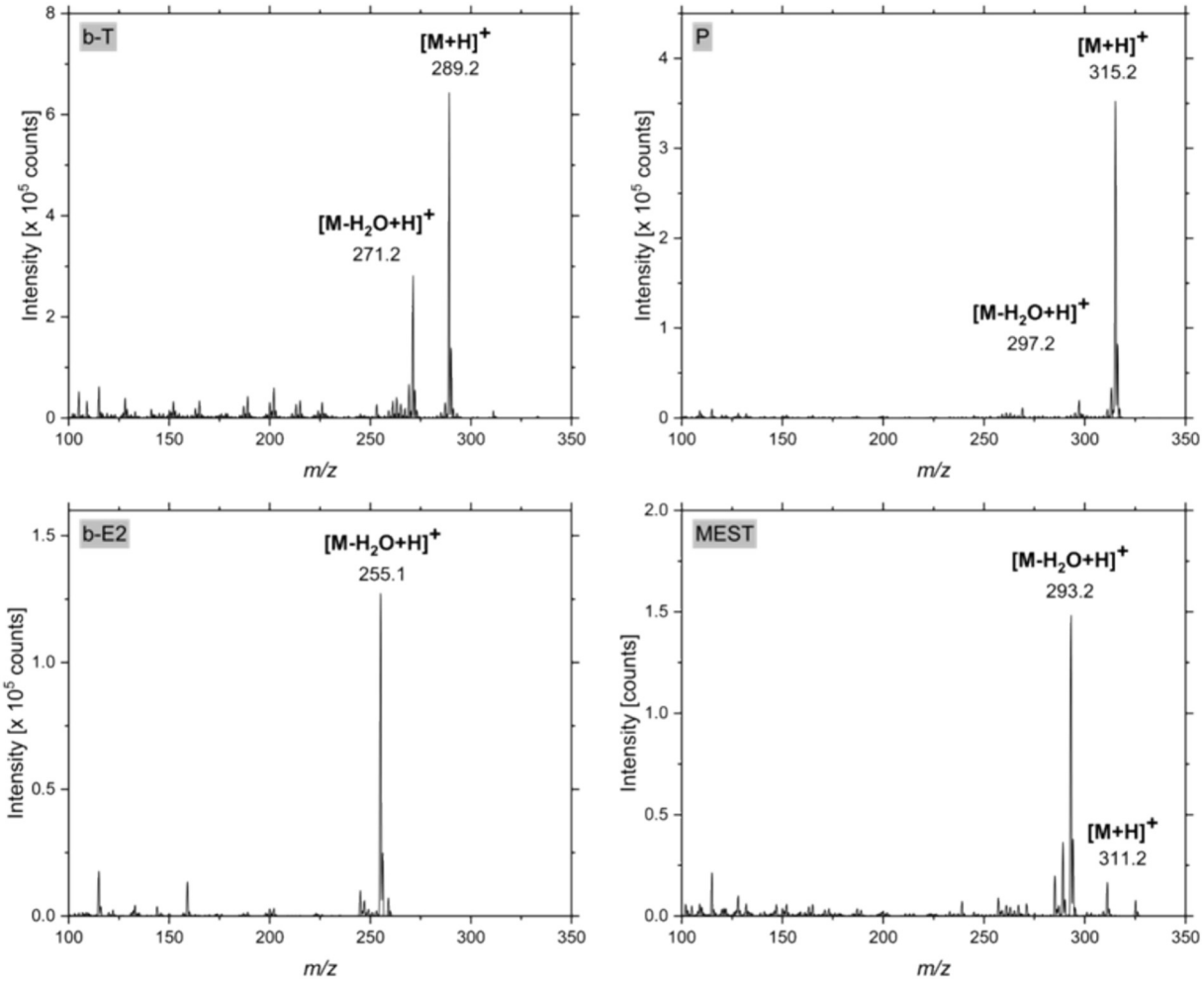

Fig. 2. Full Scan mass spectra of β-T, P, β-E2 and MEST by LC–TPI–MS.

for matrix-matched calibration solutions, accounting for pre-concentration steps in the sample preparation procedure) was studied by the determination coefficient (R [2]) of the regression function. Both repeatability (intra-day precision) and reproducibility (inter-day precision) as well as the trueness of the method were estimated using standards and spiked blank urine and meat samples at high (500 μg L^{-1}), medium (50 μg L^{-1}) and low (20 μg L^{-1}) concentration levels. Trueness was estimated as the relative deviation of the real response from the expected response based on the calibration function (%RE).

3. Results and discussion

3.1. Ionisation behaviour of estrogenic compounds by TPI

Ionisation behaviour was evaluated by flow injection analysis (FIA) of individual compounds (200 μg L^{-1}) in positive ionisation mode. Most analytes could be detected with minimal and/or without fragmentation, showing the softness of the TPI source. Earlier studies carried out by Ayala-Cabrera et al. already presented this behaviour for environmental contaminants (EPA 8610) with GC–MS. [33,34] Fig. 2 showcases, as examples, the mass spectra of β-T, P, β-E2 and MEST by TPI (+).

For most analytes (26 of 38), the protonated molecule $[M+H]^+$ was observed, being the base peak in the full scan mass spectra for 20 analytes (Table 1).

Additionally, in-source fragment ions corresponding to the loss of water moieties $[M+H\text{-}nH_2O]^+$ were occasionally observed, as the base peak of the mass spectra, as shown for β-E2 (Fig. 2). This behaviour has been widely reported for growth promoters in other API sources such as ESI [38] and APCI [39]. In some cases, such as β-CT and NEDT, the loss of two or even three water molecules were observed (Table S1). Moreover, for hexestrol and benzestrol major in-source fragmentation was observed, as the $[C_7H_7O]^+$ (107 *m/z*) ion was the base peak of the mass spectra. Although no direct relation between the chemical structure and fragmentation pattern could be concluded, some general tendencies were observed. Most androgens and progestogens were detected as $[M+H]^+$ ions, while the estrogens were usually detected as a $[M+H\text{-}nH_2O]^+$ in-source fragment ion.

Table 1
Assignment of base-peak ions generated in LC–TPI–MS under optimal conditions. Individual compounds are classified as estrogens, androgens, progestogens and stilbestrols. Further information on other ions generated is available from the supporting information.

Compound	*m/z*	Ion Assignment	Compound	*m/z*	Ion Assignment
Estrogens					
β-EEO	241.2	$[M+H\text{-}2H_2O]^+$	E1	271.0	$[M+H]^+$
α-EEO	259.2	$[M+H\text{-}H_2O]^+$	β-E2	255.1	$[M+H\text{-}H_2O]^+$
MEST	293.2	$[M+H\text{-}H_2O]^+$	α-E2	255.1	$[M+H\text{-}H_2O]^+$
EE2	279.1	$[M+H\text{-}H_2O]^+$	EED	289.2	$[M+H\text{-}H_2O]^+$
β-SOH	345.3	$[M+H]^+$	STOL	273.2	$[M+H\text{-}H_2O]^+$
Androgens					
NEDT	241.2	$[M+H\text{-}2H_2O]^+$	NAT-1O	241.2	$[M+H\text{-}2H_2O]^+$
NAT-2O	273.2	$[M+H]^+$	NATA-2O	257.2	$[M+H\text{-}H_2O]^+$
NAR-2OH	241.2	$[M+H\text{-}2H_2O]^+$	NECA	241.2	$[M+H\text{-}2H_2O]^+$
MAD	269.2	$[M+H\text{-}2H_2O]^+$	β-NT	275.2	$[M+H]^+$
17β-1-T	289.2	$[M+H]^+$	α-NT	275.2	$[M+H]^+$
METH	303.2	$[M+H]^+$	α-T	289.2	$[M+H]^+$
α-CT	323.2	$[M+H]^+$	NMD	289.2	$[M+H]^+$
MB	283.2	$[M+H\text{-}H_2O]^+$	NED	303.2	$[M+H]^+$
β-CT	323.2	$[M+H]^+$	CLAD	321.2	$[M+H]^+$
MT	303.2	$[M+H]^+$	β-T	289.2	$[M+H]^+$
17α-1-T	289.2	$[M+H]^+$	MEAD	289.2	$[M+H\text{-}H_2O]^+$
Stilbestrols					
HEX	107.1	$[C_7H_7O]^+$	BENZ	107.1	$[C_7H_7O]^+$
DE	267.1	$[M+H]^+$	DES	269.2	$[M+H]^+$
Progestogens					
P	315.2	$[M+H]^+$	NORG	313.2	$[M+H]^+$

3.2. Optimisation of LC-MS/MS methodologies

For the proper determination of target compounds using the LC–MS approach, optimisation of the analytical methodology was carried out using β-E2, E1 and EE2 as model compounds, as those are growth promoters with a well-known poor ionisation efficiency. In addition to the source-related parameters optimised for TPI and ESI sources, TPI also required optimising plasma-related parameters. For this purpose, a comparison was made between Helium and Argon, the most common plasma gases, revealing higher analyte response with argon plasma (Fig. S1a). Enhanced ionisation within the argon plasma may be explained by smaller energy gaps between the ionisation energies of analytes and Ar reactive species (Ar^m, Ar^+, Ar_2^+, <20 eV) compared to He (> 20 eV) [23,24,40]. Furthermore, the high voltage, frequency, pulse width and the discharge gas flow were optimised by a Box-Behnken design of experiments based on a response surface fitting a quadratic model (Fig. S1b).

The discharge gas frequency was evaluated from 7.5 to 12.5 kHz, with a negative correlation found between signal intensity and frequency, indicating that 7.5 kHz was the optimal value. This behaviour was already observed in GC–TPI–MS studies [34] and suggests that softer ionisation conditions (lower frequencies) may enhance the reduction of in-source fragmentation of *quasi*-molecular ions frequencies, as the energy delivery into the system is reduced. High voltage was optimised from 1.9 to 2.1 kV (due to limitations on the plasma ignition) with an optimal value at 2.0 kV. Discharge gas flow rate was evaluated from 180 to 325 mL min^{-1} and showed a response maximum at 325 mL min^{-1}. This may be explained by a greater number of reactive argon species in the system with an increased flow rate. Moreover, the pulse width was optimised from 1 to 8 μs with an optimal setpoint at 4.5 μs.

Source parameters were optimised using a design of experiments (Box-Behnken), obtaining similar trends for all the analytes. In the case of TPI, as shown in Fig. S1 for β-E2, nebuliser pressure was evaluated between 10 and 60 psi, with higher pressures yielding increased signal intensities. The vaporiser temperature, studied from 300 to 500 °C, showed an increased ionisation efficiency with higher temperatures, which may indicate that TPI ionisation for growth promoters is favourable in the absence of solvent molecules. On the other hand, the drying gas (N_2) flow rate was evaluated between 2 and 7 L min^{-1} with a slight increase of the signal intensities at higher flow rates. At the same time, the temperature showed no significant differences in the response within the tested range (200–350 °C), thus selecting 350 °C as the optimal value.

On the other hand, the AJS ESI source was optimised as well to allow for a fair comparison between the two ion sources. Information on optimisation ranges and final parameters can be found in Table S2 in the supporting information. Acquisition parameters of target compounds were optimised individually in each ionisation source. Fragmentor voltage was optimised from 50 to 200 V, and cell acceleration voltage from 1 to 7 V. Although $[M+H]^+$ ion and possible in-source fragment ions, such as $[M+H\text{-}H_2O]^+$, were monitored as potential precursor ions, the base peak selected was independent of the focussing lens settings, suggesting that the in-source fragmentation observed for certain compounds (Table 1) is mainly associated with the ionisation step. The collision energies were optimised in steps of 5 eV from 0 to 50 eV for the most abundant product ions. Full information on the transitions and optimised values is available in the supporting information (Table S3).

The in-source fragmentation and the similar structure of many growth promoters could hinder the selectivity of the method if adequate chromatographic separation is not achieved. Additionally, the mobile phase composition could have a significant influence on the ionisation efficiency of the target compounds [41,42]. For these reasons, methanol, isopropanol and acetonitrile were evaluated as organic modifiers using the LC-TPI-MS/MS analytical platform (Fig. 3a). Among them, methanol provided the highest ionisation efficiency. Besides, the influence of

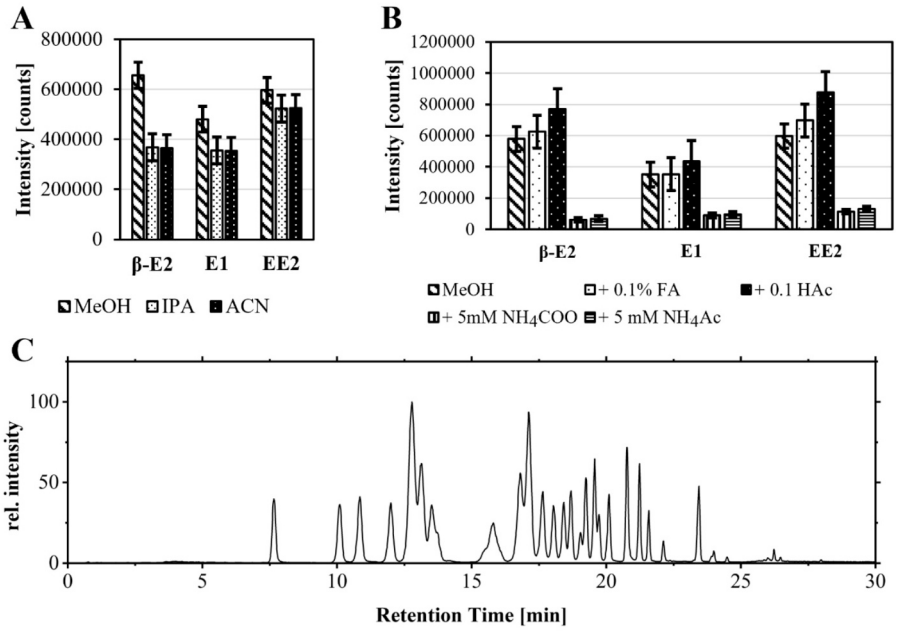

Fig. 3. A) Influence of organic solvent on the signal intensity of model compounds. B) Influence of modifiers added into the LC solvents on the signal intensity. C) Chromatogram showing the TIC of the MRM measurement of target compounds at 200 μg L^{-1}.

different additives such as formic acid, acetic acid, ammonium formate, and ammonium acetate was examined by adding them to both the organic (methanol) and aqueous (water) solvents (Fig. 3b).

The addition of the acids led to the highest analyte response, with acetic acid being the most efficient for improving the ionisation of growth promoters. This trend can be explained by the proton donating properties of acids that enhance the formation of protonated molecular ions $[M+H]^+$ and $[M+H\text{-}H_2O]^+$, which are the main species detected for the analytes of interest. On the other hand, the addition of bases (i.e., ammonium acetate and formate) led to lower signal intensities of target compounds since they act as proton acceptors. It is important to mention that growth promoters also showed a good ionisation efficiency by TPI without the addition of acidic/basic additives, which demonstrates this ion source could also show APCI-like ionisation mechanisms, allowing the protonation of the analytes after interaction with protonated water cluster ions in the gas-phase [33]. After selecting H_2O/MeOH both containing 0.1 % (*v/v*) of acetic acid as the most suitable mobile phase to improve the ionisation efficiency, the elution gradient program was optimised achieving the separation of 38 analytes in 28 min (Fig. 3c). Under optimal gradient conditions, the method allows a balance between sensitivity and selectivity as it ensure a proper ionisation efficiency and a good separation of compounds with the same *m/z* values (Fig. S2).

3.3. Comparison of the developed LC–TPI–MS/MS and LC–ESI–MS/MS methodologies

The evaluation of the developed LC–TPI–MS/MS and LC–ESI–MS/MS methodologies was based on the determination of the figures of merit using standards (instrumental) and spiked matrices (methodological) (Table 2).

In the case of the instrumental evaluation, the study was carried out at 9 concentration levels ranging from 1 to 750 μg L^{-1}. The determined instrumental limits of detection (iLOD) are significantly lower (approx.

Table 2
Median values for quality parameters with both TPI and ESI under instrumental and matrix conditions. LOD for spiked matrix includes concentration in sample preparation. A detailed comparison of each target compound can be found in the supporting information (Table S4-S10).

		Instrumental		Bovine Urine		Bovine Meat	
		TPI	ESI	TPI	ESI	TPI	ESI
LOD	μg L^{-1}	0.75	4.0	0.08	1.5	0.04	0.11
LOQ	μg L^{-1}	2.5	13	6.3	169	3.8	8
Linearity	(R [2])	0.999	0.989	0.998	0.995	0.990	0.990
Sensitivity	(L μg^{-1})	0.66	0.20	2.85	1.57	0.94	0.93
Repetability	20 μg L^{-1}	13	17	10	10	9	16
(%RSD)	50 μg L^{-1}	10	8	7	5	6	12
$n = 3$	500 μg L^{-1}	5	4	6	4	4	4
Reproducibility	20 μg L^{-1}	13	5	17	12	10	14
(%RSD)	50 μg L^{-1}	14	9	12	7	9	9
n = 3	500 μg L^{-1}	11	18	3	5	7	3
Trueness	20 μg L^{-1}	10	108	44	29	51	64
(%RE)	50 μg L^{-1}	10	20	17	15	32	32
n = 3	500 μg L^{-1}	1	6	6	11	7	7

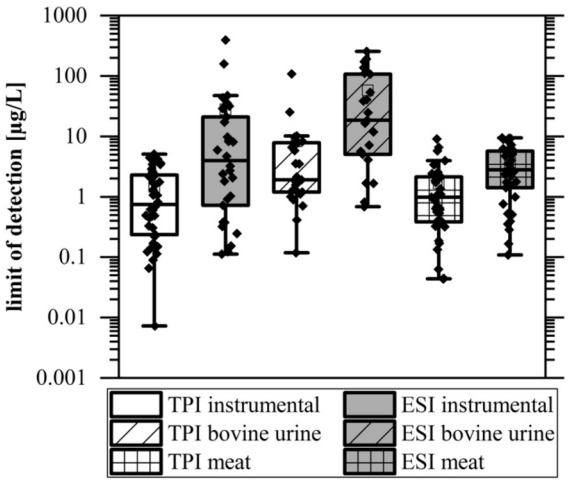

Fig. 4. Comparison of detection limits with LC–TPI–MS/MS and LC–ESI–MS/MS under instrumental and matrix conditions.

5-fold) with TPI (0.01–5.1 µg L^{-1}, median: 0.75 µg L^{-1}) compared to ESI (0.11–394 µg L^{-1}, median: 4.0 µg L^{-1}) for most analytes (Fig. 4, Table S4).

iLOD for ESI are comparable to those found in the literature. Chen et al. reported iLOD in the 1–3.8 µg L^{-1} range for ESI [22], and another study by Labadie et al. found them in the 1–2.3 µg L^{-1} range. However, both studies used significantly fewer analytes and focused on estrogens. This underlines the lower detection limits offered by TPI compared to ESI methodologies found in the literature. Further, the use of novel mobile phase additives in LC-MS, such as ammonium fluoride has also been proposed in some research for steroid analysis to enhance ionisation by ESI [43,44]. For instance, Schiffer et al. reported LOQs in the low µg L^{-1} range with LOQ usually 12-fold lower compared to TPI and 65-fold lower compared to ESI without NH_4F addition [43]. However, NH_4F comes with drawbacks as fluoride can be problematic at low pH, and salts can precipitate, requiring more intensive cleaning steps for the instrument. With TPI similar or better performance can be achieved without using salts as eluent additives, which offers major advantages in routine analysis.

A good linearity, assessed by the determination coefficient (R [2]), was achieved for almost all analytes with both ion sources, although TPI showed slightly higher R^2 (0.96–1.00, median: 0.999) than ESI (0.97–1.00, median: 0.993). Moreover, the sensitivity, estimated by the slope of the linear regression, increased when using the TPI source for most of the analytes (27 of 38). Repeatability and reproducibility of the methodologies, estimated by the relative standard deviation of intra- and inter-day precision, was similar between the two ion sources (usually <20 %, see Table 2). Moreover, with regards to instrument stability, TPI was evaluated by regular daily injections of a standard mix with intensity variations found in the range of the instrumental precision. Overall, the instrumental performance characteristics demonstrated an increased detection capability, with lower iLODs and higher sensitivity of most target compounds, using the lab-made TPI source compared to the commercially available ESI source (Table 2). Besides that, the TPI source requires maintenance schedules similar to other atmospheric pressure ionisation sources, mainly dependent on the matrix, which ensures long-term durability of the instrumental setup.

The performance characteristics of the developed methods were calculated using bovine urine, as this is a common matrix used in routine control monitoring plans. Spiked matrix was evaluated in the same concentration range as the instrumental validation. As can be seen in Fig. 4, method limits of detection (mLOD) were lower (approx. 9-fold) with TPI (4.7–4333 ng L^{-1}, median: 76 ng L^{-1}) than with ESI (27–18,497 ng L^{-1}, median: 1536 ng L^{-1}). The proposed LC–TPI–MS/MS method fulfils the detection criteria as set by the EURL for growth promoters of 100–2000 ng L^{-1} in urine matrix. The LOD of the developed LC-TPI-MS/MS method, as expressed in the amount on column, ranges from 23.7 fg on column to 21.7 pg. In the literature, several methodologies for the determination of growth promoters and veterinary drug residues in various matrices have been published. These methods follow Commission Decision 2002/657/EC [8] for the calculation of performance characteristics. To determine when a sample deviates from zero for a forbidden substance, the decision limit (CCα) is used for forbidden substances. The CCα is typically 10–50 % higher than the limit of detection (LOD). This increase is caused by the use of the false positive (α) error probabilities. Since the LOD is not determined for these published methods, we compared the LOD with the CCα in this study. Blokland et al. proposed a method for analysis of steroid hormones in urine based on LC–ESI–MS/MS with LOD analogous CCα-values ranging from 3.3 pg on column to 29.9 ng on column [14]. A method published by da Silva et al. reached CCα of 0.2 to 6.8 pg on column [45] whilst an approach by Kaklamanos et al. achieved 0.9 to 4.2 pg on column [46]. However, these methodologies screen a smaller number of analytes and require significantly higher injection volumes. The observed linearity was acceptable ($R^2 > 0.99$) for most analytes however, a split in the calibration curve for high and low concentration levels is necessary for several analytes like α-CT, β-T or MEAD. Nevertheless, higher linearities (R [2]) were observed with TPI (TPI, mean: 0.998; ESI, mean: 0.995). The sensitivity, as already proven for instrumental conditions, is in general higher with TPI (0.09–152, median 2.9) than with ESI (0.02–458, median 1.6), although some exceptions such as β-NT, NMD and 17α-1-T exist. Repeatability, reproducibility (RSD, %) and trueness (RE, %) are comparable between the two sources (< 20 %, with a median of 10 %). With these experiments TPI demonstrates an increase in sensitivity and detection capability for the matrix as well. Furthermore, the matrix effect (ME, %) was estimated as the difference in the response between the spiked extract and the corresponding standard for both ion sources, revealing a signal decrease in both cases (Fig. 5). With TPI a lower ME was observed with a signal decrease by −36 to −9 % (median: −28 %) compared to ESI with ME between −61 to −29 % (median: −48 %). The lower ME values achieved when using the TPI source might be due to the gas-phase ionisation mechanisms which is less prone to be affected by high abundant polar matrix components present in the urine. Benijts et al. discussed strategies to reduce matrix effects and demonstrated that the addition of modifiers, and especially salts, to HPLC eluents significantly reduces ion suppression and enhancement [16]. However, as already discussed, adding salts to eluents is unfavorable for routine methods.

In addition to evaluation in bovine urine, bovine meat samples were

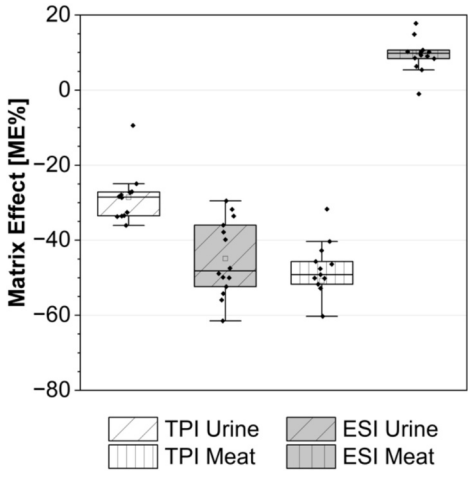

Fig. 5. Comparison of matrix effects (ME, %) of bovine urine and meat matrices with TPI and ESI.

also considered, as they are typically used when urine is unavailable. The analytical procedure was similar to the one carried out with bovine urine. Detection limits found with TPI ranged from 0.04 μg L^{-1} to 9.1 μg L^{-1} (median: 0.85 μg L^{-1}) and were lower (approx. 3-fold) than those found with ESI (0.11 μg L^{-1}–9.3 μg L^{-1}, median: 2.3 μg L^{-1}). Furthermore, benzestrol and α-chlorotestosterone could not be detected by ESI, which could be detected with TPI at 9.1 and 4.0 μg L^{-1}, respectively. It must be mentioned that the meat matrix shows a greater influence on the detection limits, as these are, in general, higher than those determined for the urine matrix. For instance, in the case of MEAD, the matrix effect resulted in a 19 % signal reduction in meat, whereas in urine, a 1 % signal reduction was observed. In general, the meat matrix led to stronger ion suppression with TPI and slight signal enhancement with ESI (Fig. 5). Contrary to what happens with urine analysis, meat matrix components are more liphophilic which might be more efficiently ionized in the TPI source compared with ESI which is more affected by hydrophilic matrix components. The ME found for TPI was in the −92 to −32 % range (median: −49 %) and for ESI, it was in the −1–21 % range (median: 10 %). Overall, despite the significant matrix suppression observed for some compounds in the TPI source, the method offers low detection limits ranging from 0.2 pg on column (0.44 ng kg^{-1}) to 49 pg on column (91 ng kg^{-1}) with a median value of 4.3 pg on column (9.8 ng kg^{-1}). A method published by van Tricht et al. found CCα in a range of 0.1–7 μg/kg [17], whilst Antignac et al. found detection limits of 0.04 to 0.07 μg kg^{-1} [47] although the method focussed on corticosteroids instead of anabolic steroids. The linearity was comparable with both ion sources (TPI: 0.992, ESI: 0.990), as were repeatability and reproducability (RSD < 15 % above the LOQ) and trueness (RE < 35 % at 50 μg L^{-1} and < 8 % at 500 μg L^{-1}). Besides, TPI showed higher sensitivity (median slope: 0.46) compared to ESI (median slope: 0.30).

Overall, TPI demonstrated increased performance over ESI in LC-MS experiments, even without the addition of signal-enhancing modifiers. With a low LOD and high linearity, precision (both intra- and inter-day), and trueness, a reliable methodology for determining growth promoters was established.

3.4. Occurrence of growth promoting compounds in real samples

The developed and tested LC-TPI-MS/MS method was applied to the analysis of growth promoters in anonymised samples. For this purpose, urine and meat extracts provided by the EURL Growth Promoters were spiked at different concentrations. Table 3 summarises the quantification results. In all four urine samples, analytes were found. While in U1 and U2, the concentrations found were generally higher, U3 and U4 demonstrate the ability of TPI to quantify low concentrations in real urine samples. Based on this study, a larger validation study using a more extensive and representative sample collection is planned when the method will be applied in a routine setting. Fig. S3 showcases the extracted ion chromatograms of quant-MRM transitions in sample U2. Generally, the standard deviation found in spike samples was acceptable and in line with the method validation. Whilst in samples U2 and U4 several compounds were found, U1 and U3 contained only one analyte. On the other hand, in the spiked meat samples, analytes were found in M1, M2, and M3. In sample M4 no analytes were found. M1 and M2 contained several analytes in the low μg/kg range, whilst in M3 only β-CT was found. Overall, meat showed greater matrix influence with the spiked samples.

In sample M4 no analytes were found. M1 and M2 contained several analytes in the low μg/kg range, whilst in M3 only β-CT was found. Overall, meat showed greater matrix influence with the spiked samples.

4. Conclusions

TPI was introduced as an alternative approach for analysing growth promoters to enhance analytical performance and reduce common issues, especially the low ionisation efficiency, observed when using ESI. Moreover, it also reduced strong matrix effect in the case of hydrophilic matrices such as urine although it seems to be more affected by matrix components when analysising more liphophilic samples such as meat. TPI offers soft ionisation with low in-source fragmentation, and analytes are usually detected with minimal fragmentation to the carbon structure. Androgens and progestogens are typically detected by the protonated molecular ion $[M+H]^+$ while estrogens are usually detected by the $[M–H_2O+H]^+$ ion. The soft ionisation observed increases the selectivity and sensitivity of the analysis. A careful optimisation of plasma parameters showed the highest signal intensities with 2.0 kV applied by 7.5 kHz pulses of 4.5 μs pulse width. Argon showed higher intensities compared to Helium as discharge gas and should be introduced at higher flow rates (325 mL min^{-1}). A comparison with the introduced LC–TPI–MS/MS approach demonstrated increased performance over LC-ESI-MS/MS, reducing major drawbacks such as matrix effects. TPI offers lower detection limits for both instrumental conditions and in matrix (bovine urine and meat). The sensitivity observed with TPI was increased compared to ESI. Linearity, repeatability, reproducability and trueness were comparable or slightly better in TPI. Overall, the better analytical performance of TPI was proven. Therefore, TPI can offer an alternative approach for the screening of growth promoters. However, the source is not yet commercialised, and the set-up is more complicated compared to ESI as it requires additional parts and gas flows. Therefore, a decision must be made between superior performance and an off-the-

Table 3
Levels of growth promoters found in spiked bovine urine (U1 – U4) and meat samples (M1 – M4) with LC-TPI-MS/MS. Not all analytes were present in all samples. Concentrations are calculated based on the original volume of the urine sample or the mass of the meat sample.

β	U1 [μg L^{-1}]	U2 [μg L^{-1}]	U3 [μg L^{-1}]	U4 [μg L^{-1}]	M1 [μg/kg]	M2 [μg/kg]	M3 [μg/kg]	M4 [μg/kg]
17α-1-T	< LOD	57.2 ± 6.66	0.70 ± 0.02	0.55 ± 0.05	< LOD	< LOD	< LOD	< LOD
17β-1-T	< LOD	< LOD	< LOD	0.24 ± 0.003	< LOD	< LOD	< LOD	< LOD
α-T	< LOD	< LOD	< LOD	1.64 ± 0.10	< LOD	< LOD	< LOD	< LOD
α-CT	< LOD	< LOD	< LOD	< LOD	18.3 ± 1.10	< LOD	< LOD	< LOD
A-EEO	< LOD	< LOD	< LOD	< LOD	< LOD	8.31 ± 1.65	< LOD	< LOD
β-CT	< LOD	45.2 ± 6.66	< LOD	< LOD	< LOD	< LOD	9.66 ± 2.04	< LOD
β-E2	< LOD	< LOD	< LOD	1.12 ± 0.06	< LOD	< LOD	< LOD	< LOD
β-NT	< LOD	< LOD	< LOD	0.61 ± 0.12	< LOD	< LOD	< LOD	< LOD
β-T	< LOD	46.1 ± 5.51	< LOD	1.25 ± 0.44	< LOD	9.47 ± 0.85	< LOD	< LOD
B-EEO	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
E1	250 ± 44	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
EE2	< LOD	< LOD	< LOD	< LOD	2.32 ± 0.08	< LOD	< LOD	< LOD
MAD	< LOD	< LOD	< LOD	< LOD	5.76 ± 0.63	< LOD	< LOD	< LOD
MEAD	< LOD	237 ± 14	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
MEST	< LOD	< LOD	< LOD	< LOD	13.0 ± 0.74	0.47 ± 0.02	< LOD	< LOD
NEDT	< LOD	< LOD	< LOD	< LOD	< LOD	7.84 ± 1.66	< LOD	< LOD
NMD	< LOD	< LOD	< LOD	0.78 ± 0.10	< LOD	< LOD	< LOD	< LOD

shelf solution, depending on the specific requirements of the analysis. Nevertheless, further research into plasma-based ionisation sources will most likely lead to easier to-use and less complicated set-ups, since neither high voltage nor argon is new to the analytical laboratory. Furthermore, TPI is an ion sources increasing the simplicity and sensitivity compared to earlier developed versions of plasma-based ion sources and showcases the trend towards more user-friendly plasma sources.

CRediT authorship contribution statement

Sebastian Löbbecke: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Florian Stappert:** Writing – review & editing, Investigation, Formal analysis. **Florian Uteschil:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Marco H. Blokland:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Oliver J. Schmitz:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Ane Arrizabalaga-Larrañaga:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Juan F. Ayala-Cabrera:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2025.115603.

Data availability

Data will be made available on request.

References

[1] A. Raun, R. Preston, History of hormonal modifier use, OSU Implant Symposium (1997) 1–9.
[2] A. Raun, R. Preston, History of diethylstilbestrol use in cattle, J. Anim. Sci. 80 (2002) 1–7.
[3] S.N. Al-Dobaib, H. Mousa, Benefits and risks of growth promoters in animal production, J. Food Agric. Environ. 7 (2009) 202–208.
[4] Opinion of the scientific panel on contaminants in the food chain (CONTAM) related to hormone residues in bovine meat and meat products, EFSA J. 5 (2007) 510, https://doi.org/10.2903/j.efsa.2007.510.
[5] Council Directive 96/22/EC of 29 April 1996 concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of ß-agonists. OJ L 125 1996, 23.5, 3–9.
[6] Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products, amending Regulations (EC) No 999/2001, (EC) No 396/2005, (EC) No 1069/2009, (EC) No 1107/2009, (EU) No 1151/2012, (EU) No 652/2014, (EU) 2016/429 and (EU) 2016/2031 of the European Parliament and of the Council, Council Regulations (EC) No 1/2005 and (EC) No 1099/2009 and Council Directives 98/58/EC, 1999/74/EC, 2007/43/EC, 2008/119/EC and 2008/120/EC, and repealing Regulations (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council, Council Directives 89/608/EEC, 89/662/EEC, 90/425/EEC, 91/496/EEC, 96/23/EC, 96/93/EC and 97/78/EC and Council Decision 92/438/EEC (Official Controls Regulation). OJ L 95 2017, 7.4, 1–142.
[7] M. Gonzalez Ronquillo, J.C. Angeles Hernandez, Antibiotic and synthetic growth promoters in animal diets: review of impact and analytical methods, Food Control 72 (2017) 255–267, https://doi.org/10.1016/j.foodcont.2016.03.001.
[8] Commission Implementing Regulation (EU) 2021/808 of 22 March 2021 on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC. OJ L 180 2021, *21.5*, 84–109.
[9] B. Le Bizec, P. Marchand, D. Maume, F. Monteau, F. Andr, Monitoring anabolic steroids in meat-producing animals. Review of current hyphenated mass spectrometric techniques, Chromatographia 59 (2004) S3–S11, https://doi.org/10.1365/s10337-004-0245-5.
[10] E.A. Daeseleire, A. Guesquière, C.H. Van Peteghem, Multiresidue analysis of anabolic agents in muscle tissues and urines of cattle by GC-MS, J. Chromatogr. Sci. 30 (1992) 409–414, https://doi.org/10.1093/chromsci/30.10.409.
[11] M. Thevis, W. Schänzer, Mass spectrometry in sports drug testing: structure characterization and analytical assays, Mass Spectrom. Rev. 26 (2007) 79–107, https://doi.org/10.1002/mas.20107.
[12] F. Moussa, S. Mokh, S. Doumiati, B. Barboni, N. Bernabò, M. Al Iskandarani, LC-MS/MS method for the determination of hormones: validation, application and health risk assessment in various bovine matrices, Food Chem. Toxicol. 138 (2020) 111204, https://doi.org/10.1016/j.fct.2020.111204.
[13] M.R. Anari, R. Bakhtiar, B. Zhu, S. Huskey, R.B. Franklin, D.C. Evans, Derivatization of ethinylestradiol with dansyl chloride to enhance electrospray ionization: application in trace analysis of ethinylestradiol in rhesus monkey plasma, Anal. Chem. 74 (2002) 4136–4144, https://doi.org/10.1021/ac025712h.
[14] M.H. Blokland, E.F. van Tricht, L.A. van Ginkel, S.S. Sterk, Applicability of an innovative steroid-profiling method to determine synthetic growth promoter abuse in cattle, J. Steroid Biochem. Mol. Biol. 174 (2017) 265–275, https://doi.org/10.1016/j.jsbmb.2017.10.007.
[15] I. Athanasiadou, Y.S. Angelis, E. Lyris, C. Georgakopoulos, Chemical derivatization to enhance ionization of anabolic steroids in LC-MS for doping-control analysis, TrAC 42 (2013) 137–156, https://doi.org/10.1016/j.trac.2012.10.003.
[16] T. Benijts, R. Dams, W. Lambert, A. Leenheer, Countering matrix effects in environmental liquid chromatography-electrospray ionization tandem mass spectrometry water analysis for endocrine disrupting chemicals, J. Chromatogr. A 1029 (2004) 153–159, https://doi.org/10.1016/j.chroma.2003.12.022.
[17] F. van Tricht, M. Essers, M. Groot, S. Sterk, M. Blokland, L. van Ginkel, A fast quantitative multi-analyte method for growth promoters in bovine meat using bead-disruption, 96-well SPE clean-up and narrow-bore UHPLC-MS/MS analysis, Food Anal. Methods 11 (2018) 2206–2217, https://doi.org/10.1007/s12161-018-1164-7.
[18] Z. Wang, Z. Shi, C. Xi, G. Wang, S. Cao, L. Zhang, B. Tang, Z. Mu, Simultaneous detection of 15 antibiotic growth promoters in bovine muscle, blood and urine by UPLC-MS/MS, Food Addit. Contam. Part A 34 (2017) 2144–2154, https://doi.org/10.1080/19440049.2017.1382724.
[19] S.J. Hird, B.P.-Y. Lau, R. Schuhmacher, R. Krska, Liquid chromatography-mass spectrometry for the determination of chemical contaminants in food, TrAC 59 (2014) 59–72, https://doi.org/10.1016/j.trac.2014.04.005.
[20] S.J. Soldin, O.P. Soldin, Steroid hormone analysis by tandem mass spectrometry, Clin. Chem. 55 (2009) 1061–1066, https://doi.org/10.1373/clinchem.2007.100008.
[21] J.-F. Hsu, Y.-C. Chang, T.-H. Chen, L.-C. Lin, P.-C. Liao, Evaluation of electrospray ionization and atmospheric pressure chemical ionization for simultaneous detection of estrone and its metabolites using high-performance liquid chromatography/tandem mass spectrometry, J. Chromatogr. B 860 (2007) 49–56, https://doi.org/10.1016/j.jchromb.2007.10.014.
[22] H.-C. Chen, H.-W. Kuo, W.-H. Ding, Determination of estrogenic compounds in wastewater using liquid chromatography-tandem mass spectrometry with electrospray and atmospheric pressure photoionization following desalting extraction, Chemosphere 74 (2009) 508–514, https://doi.org/10.1016/j.chemosphere.2008.09.089.
[23] J.F. Ayala-Cabrera, L. Montero, S.W. Meckelmann, F. Uteschil, O.J. Schmitz, Review on atmospheric pressure ionization sources for gas chromatography-mass spectrometry. Part I: current ion source developments and improvements in ionization strategies, Anal. Chim. Acta 1238 (2023) 340353, https://doi.org/10.1016/j.aca.2022.340353.
[24] A. Pape, O.J. Schmitz, Dielectric barrier discharge in mass spectrometry – an overview over plasma investigations and ion sources applications, TrAC 170 (2024) 117420, https://doi.org/10.1016/j.trac.2023.117420.
[25] A. Michels, S. Tombrink, W. Vautz, M. Miclea, J. Franzke, Spectroscopic characterization of a microplasma used as ionization source for ion mobility

spectrometry, Spectrochim. Acta Part B: At. Spectrosc. 62 (2007) 1208–1215, https://doi.org/10.1016/j.sab.2007.08.004.
[26] N. Na, M. Zhao, S. Zhang, C. Yang, X. Zhang, Development of a dielectric barrier discharge ion source for ambient mass spectrometry, J. Am. Soc.Mass Spectrom. 18 (2007) 1859–1862, https://doi.org/10.1016/j.jasms.2007.07.027.
[27] H. Yue, F. He, Z. Zhao, Y. Duan, Plasma-based ambient mass spectrometry: recent progress and applications, Mass Spectrom. Rev. 42 (2023) 95–130, https://doi.org/10.1002/mas.21712.
[28] J.D. Harper, N.A. Charipar, C.C. Mulligan, X. Zhang, R.G. Cooks, Z. Ouyang, Low-temperature plasma probe for ambient desorption ionization, Anal. Chem. 80 (2008) 9097–9104, https://doi.org/10.1021/ac801641a.
[29] X. Ding, Y. Duan, Plasma-based ambient mass spectrometry techniques: the current status and future prospective, Mass Spectrom. Rev. 34 (2015) 449–473, https://doi.org/10.1002/mas.21415.
[30] S. Martínez-Jarquín, R. Winkler, Low-temperature plasma (LTP) jets for mass spectrometry (MS): ion processes, instrumental set-ups, and application examples, TrAC 89 (2017) 133–145, https://doi.org/10.1016/j.trac.2017.01.013.
[31] D. Brecht, F. Uteschil, O.J. Schmitz, Development of an inverse low-temperature plasma ionization source for liquid chromatography/mass spectrometry, Rapid Commun. Mass Spectrom. 35 (2021) e9071, https://doi.org/10.1002/rcm.9071.
[32] S. Brandt, F.D. Klute, A. Schütz, U. Marggraf, C. Drees, P. Vogel, W. Vautz, J. Franzke, Flexible microtube plasma (FμTP) as an embedded ionization source for a microchip mass spectrometer Interface, Anal. Chem. 90 (2018) 10111–10116, https://doi.org/10.1021/acs.analchem.8b01493.
[33] J.F. Ayala-Cabrera, J. Turkowski, F. Uteschil, O.J. Schmitz, Development of a tube plasma ion source for gas chromatography-mass spectrometry analysis and comparison with other atmospheric pressure ionization techniques, Anal. Chem. 94 (2022) 9595–9602, https://doi.org/10.1021/acs.analchem.2c00582.
[34] S. Löbbecke, A. Pape, L. Montero, F. Uteschil, J.F. Ayala-Cabrera, O.J. Schmitz, Improving the reliability of phthalate esters analysis in water samples by gas chromatography-tube plasma ionization-high-resolution mass spectrometry (GC-TPI-HRMS), Talanta 285 (2024) 127388, https://doi.org/10.1016/j.talanta.2024.127388.
[35] J.F. Ayala-Cabrera, L. Montero, T. Sahlabji, O.J. Schmitz, Comprehensive two-dimensional gas chromatography with flow modulator coupled via tube plasma ionization to an atmospheric pressure high-resolution mass spectrometer for the analysis of vermouth volatile profile, Anal. Bioanal. Chem. 415 (2023) 2561–2573, https://doi.org/10.1007/s00216-023-04688-6.
[36] A. Pape, Improvement of Atmospheric Pressure Ion Sources for Mass Spectrometry, DuEPublico: Duisburg-Essen Publications online, University of Duisburg-Essen, Germany, 2024 accessed 25th september 2025.
[37] T. Pihlström, A.R. Fernández-Alba, C. Ferrer, M.E. Poulsen, B. Hardebusch, M. Anastassiades, Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed: *SANTE/11312/2021 (V2)*, 2021, pp. 1–51.
[38] B. Drotleff, M. Hallschmid, M. Lämmerhofer, Quantification of steroid hormones in plasma using a surrogate calibrant approach and UHPLC-ESI-QTOF-MS/MS with SWATH-acquisition combined with untargeted profiling, Anal. Chim. Acta 1022 (2018) 70–80, https://doi.org/10.1016/j.aca.2018.03.040.
[39] L. Chu, N. Li, J. Deng, Y. Wu, H. Yang, W. Wang, D. Zhou, H. Deng, LC-APCI+-MS/MS method for the analysis of ten hormones and two endocannabinoids in plasma and hair from the mice with different gut microbiota, J. Pharm. Biomed. Anal. 185 (2020) 113223, https://doi.org/10.1016/j.jpba.2020.113223.
[40] J. Kratzer, Z. Mester, R.E. Sturgeon, Comparison of dielectric barrier discharge, atmospheric pressure radiofrequency-driven glow discharge and direct analysis in real time sources for ambient mass spectrometry of acetaminophen, Spectrochim. Acta Part B: At. Spectrosc. 66 (2011) 594–603, https://doi.org/10.1016/j.sab.2011.06.005.
[41] K.M. Lee, S.M. Han, H.-J. Lee, M. Kang, T.Y. Jeong, J. Son, H. Min, S. Cha, H.B. Oh, W.K. Oh, et al., Influence of mobile phase composition on the analytical sensitivity of LC-ESI-MS/MS for the concurrent analysis of bisphenols, parabens, chlorophenols, benzophenones, and alkylphenols, Environ. Res. 221 (2023) 115305, https://doi.org/10.1016/j.envres.2023.115305.
[42] J.F. Ayala-Cabrera, F. Javier Santos, E. Moyano, Negative-ion atmospheric pressure ionisation of semi-volatile fluorinated compounds for ultra-high-performance liquid chromatography tandem mass spectrometry analysis, Anal. Bioanal. Chem. 410 (2018) 4913–4924, https://doi.org/10.1007/s00216-018-1138-z.
[43] L. Schiffer, F. Shaheen, L.C. Gilligan, K.-H. Storbeck, J.M. Hawley, B.G. Keevil, W. Arlt, A.E. Taylor, Multi-steroid profiling by UHPLC-MS/MS with post-column infusion of ammonium fluoride, J. Chromatogr. B 1209 (2022) 123413, https://doi.org/10.1016/j.jchromb.2022.123413.
[44] M. Juck, W. Long, Determination of Hormones in Serum by LC/MS/MS Using Agilent Bond Elut Plexa SPE: Application Note; Santa Clara, CA, 2017 (accessed 25th september 2025).
[45] M.C. Da Silva, T.F. Borgati, D.G. Rocha, M.A.G. Lana, A.F. Faria, A quantitative and confirmatory method employing liquid chromatography coupled to hybrid high-resolution mass spectrometry and QuEChERS for the determination of thirty-seven growth promoter residues in bovine urine, J. Agric. Food Chem. 69 (2021) 7990–7996, https://doi.org/10.1021/acs.jafc.1c02286.
[46] G. Kaklamanos, G. Theodoridis, T. Dabalis, Determination of anabolic steroids in bovine urine by liquid chromatography-tandem mass spectrometry, J. Chromatogr. B 877 (2009) 2330–2336, https://doi.org/10.1016/j.jchromb.2009.03.033.
[47] J.P. Antignac, B. Le Bizec, F. Monteau, F. Poulain, F. Andre, Multi-residue extraction-purification procedure for corticosteroids in biological samples for efficient control of their misuse in livestock production, J. Chromatogr. B 757 (2001) 11–19, https://doi.org/10.1016/S0378-4347(00)00626-5.