



Short communication

Exploring the potential of using ion mobility-mass spectrometry to separate matrix interferences from analytes in food control

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ABSTRACT

During residue analysis in complex matrices for food safety purposes, interfering signals can sometimes overlap with those of the analyte of interest. Access to an additional separation dimension besides chromatographic and mass separation, such as ion mobility, can aid in removing interfering signals, allowing for correct analyte identification in these cases. In our laboratory, during routine LC–MS/MS analysis of liver samples for growth promoter residues, an interfering signal was found that matches the retention time and m/z values for stanozolol, a synthetic anabolic steroid. In the present work, the performance of a liquid chromatography coupled to ion mobility mass spectrometry (LC–IM–MS) method has been evaluated to study whether this LC–MS/MS false positive in liver samples could be eliminated by LC–IM–MS analysis. A cyclic ion mobility system already allowed the separation of stanozolol from the interfering peak after only one pass, showing a significant improvement compared to the conventional LC–MS/MS method. Additionally, collisional cross section (CCS) values were calculated and successfully compared with those from literature for identification purposes, eventually allowing both the identification and quantification of stanozolol in this complex matrix.

1. Introduction

Routine monitoring of e.g. pesticide, mycotoxin, and veterinary drug residues are important to maintain a safe food supply [1]. In recent years there has been a trend in the development of the analytical methods employed, which shifts from specific targeted to untargeted multi-residue methods to increase efficiency and lower costs [2–4]. Although liquid chromatography high-resolution mass spectrometry (LC–HRMS) is considered to be the technique of choice for multi-residue monitoring due to its untargeted nature [5], the main analytical technique used for residue monitoring for food safety is still based on targeted, liquid chromatography-tandem mass spectrometry (LC–MS/MS) [6]. However, due to the ever-increasing numbers of analytes and matrices that need to be analysed, the rate at which matrix interferences become problematic in the targeted methods increases, resulting more often in poor sensitivity or, in some cases, even false positive or false negative findings [7]. Multi-residue methods potentially benefit from having access to an additional separation dimension, allowing separation of interferences and analytes of interest without changing sample preparation procedures or chromatographic conditions.

Ion mobility spectrometry (IMS) allows the separation of molecules

based on their three-dimensional structure. IMS can be integrated into existing LC–MS methods, thus providing an additional separation dimension (LC–IM–MS). Different types of IMS platforms have been developed, such as drift tube IMS (DTIMS), travelling wave IMS (TWIMS) and trapped IMS (TIMS) [8,9]. Generally, ions are introduced in a drift tube and moved forward under the influence of an electric field. Their mobility in the drift tube depends on their shape, size, and charge, and the residence time in the drift tube is known as the drift time [10]. A molecule collisional cross section (CCS) can be determined from the drift time value. The CCS value is corrected for the strength of the applied electric field, the drift gas pressure, and the temperature. This theoretically results in the same CCS for each compound using different instruments (DTIMS, TWIMS, or TIMS) or instrument settings [9]. EU Regulation 2021/808 establishes the use of identification points for the confirmation of the identity of substances in residue analysis. When IMS is coupled to liquid chromatography mass spectrometry, the CCS value can potentially be used as an additional identification point, besides the retention time and m/z values [11–13]. IMS is especially beneficial when analysing compounds with small structural differences, such as isomers [9], or when matrix interferences overlap with analyte signals. The present study investigated the potential of a new ion mobility

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instrument [14], which uses TWIMS in a cyclic configuration (cIMS) for removing a matrix interferent which complicates unambiguous confirmation of stanozolol abuse, commonly seen during targeted food control analyses using LC–MS/MS, i.e. when analysing liver tissue for the presence of (forbidden) steroids.

2. Experimental section

2.1. Chemicals and reagents

LC–MS grade acetonitrile, methanol and formic acid were purchased from Actu-All Chemicals (Oss, the Netherlands), glacial acetic acid from Merck (Burlington, MA, USA), and ammonium formate and dimethylsulfoxide (DMSO) from Sigma-Aldrich (Saint Louis, MO, USA). Stanozolol was obtained from EDQM (Strasbourg, France). Deionized water (Produced with a Milli-Q Reference A + system; Merck KGaA, Darmstadt, Germany) was used for all experiments.

2.2. Sample preparation

Poultry liver samples were prepared according to an extension of our internal procedure used to analyze a selection of growth promoters in muscle, poultry liver, water, and fish which is based on van Tricht et al. [15]. During this procedure, samples are extracted using a beadruptor (Omni international 24) (Omni international, Kennesaw, GA). Briefly, a layer of beads is added to 7 mL sample tubes and next, 500 mg of homogenized liver sample and 1 mL extraction solvent (acetonitrile:water, 60:40 v/v) is added to the tube. The beadruptor extraction is then performed at a speed of 5.65 m s⁻¹, a cycle time of 0.75 min, pause dwell time of 0.5 min and two cycles are performed in total. Next, the tubes are centrifuged for 10 min at 3452 g and the supernatant is transferred to a clean glass tube and 2 mL of water is added. Afterwards, a 96 well SPE plate (Oasis HLB, 60 mg, 60 µm) (Waters Corporation, Milford, MA, USA) is conditioned using 1 mL of methanol followed by 1 mL of water. The whole extract is transferred from the glass tube into one of the wells and then washed with 1 mL of water and dried. Next, the well is washed using 1 mL of a sequence of washing solutions with drying in between (1: methanol:water:acetic acid (60:38:2 v/v/v), 2: water:acetonitrile:acetic acid, (78:20:2 v/v/v), 3: water:acetonitrile (80:20 v/v), 4: water:acetonitrile:ammonia (82:10:8 v/v/v), 5: methanol:water:ammonia (50:42:8 v/v/v) and 6: methanol:water (60:40 v/v)). Then 20 µL of DMSO is pipetted in the well of a collection plate (96-well collection plate for Waters Acquity UPLC I-Class, 2 mL) (Waters Corporation, Milford, MA, USA) as a keeper and the extracts are eluted from the SPE plate using 1 mL of acetonitrile. The extracts in the collection plate are then evaporated until only the DMSO keeper is left. Finally, 50 µL of reconstitution solvent (methanol:water, 10:90 v/v) is added and the plate is sealed. Liver extracts were spiked with stanozolol using 5 µL of a 350 µg L⁻¹ standard solution of stanozolol in methanol, which corresponds to a concentration level of 3.5 µg kg⁻¹ in the original matrix.

2.3. Chromatography

Reversed-phase chromatographic separation was performed on an Acquity UPLC H-Class PLUS Bio System (Waters Corporation, Milford, MA, USA) for LC–IM–TOF-MS experiments and on an Acquity UPLC I-Class System (Waters Corporation, Milford, MA, USA) for LC–MS/MS experiments, both using an Acquity UPLC BEH C18 column (100 mm x 1.0 mm, 1.7 µm I.D.) (Waters Corporation, Milford, MA, USA). The mobile phase components for both experiments consisted of water:acetonitrile:formic acid:ammonium formate 1 M, 900:100:0.02:2, v/v/v/v (A) and acetonitrile:water:formic acid:ammonium formate 1 M, 900:100:0.02:2, v/v/v/v (B). A gradient profile was used starting with 20 % B for 0.2 min, moving to 30 % B at 3.2 min, 50 % B at 7.5 min, 100 % B at 7.6 min held until 8.6 min, moving back to 20 % B at 8.7 min held until 10 min. The flow was 0.15 mL min⁻¹ for the whole gradient. The

column temperature was set to 60 °C.

2.4. Mass spectrometry

For LC–IM–TOF-MS experiments, the UPLC system was interfaced with an IM–TOF-MS (select series Cyclic IMS instrument, Waters Corporation, Milford, MA, USA) using an electrospray ionization source and operated in positive ion mode (2.5 kV). The TOF-MS was calibrated using a sodium iodide solution. The instrument was operated in V-mode using a 1 s scanning rate. The cone was set to 40 V, the source offset to 10 V, the cone gas flow to 50 L hour⁻¹ and the nebulizer gas to 6 bar. The desolvation gas flow and temperature were set to 400 L hour⁻¹ and 250 °C. The TOF scan range was set to 50 – 1200 m/z. For LC–MS/MS experiments, the UPLC system was interfaced to a triple quadrupole MS system (Xevo TQXS, Waters Corporation, Milford, MA, USA) using an electrospray ionization source and operated in positive ion mode (3.0 kV). The cone was set to 20 V, the source offset to 50, the cone gas flow to 150 L hour⁻¹ and the nebulizer gas to 7 bar. The desolvation gas flow and temperature were set to 800 L hour⁻¹ and 400 °C.

2.5. Cyclic ion mobility and CCS calibration

The instrument was calibrated in positive ion mode using direct infusion of Major Mix IMS calibration solution (Waters Corporation, Milford, MA, USA). The following compounds were selected for calibration curve construction: sulfaguandine (m/z 215.0597, CCS 146.8 Å²), sulfadimethoxine (m/z 311.0809, CCS 168.4 Å²), Val-Tyr-Val (m/z 380.2180, CCS 191.7 Å²), verapamil (m/z 455.2904, CCS 208.8 Å²) and terfenadine (m/z 472.3210, CCS 228.7 Å²). Data were gathered using up to three passes to determine CCS values of knowns and unknowns, i.e. analyte of interest and interfering compound. A logarithmic calibration curve was constructed according to Mccullagh et al. [16] [17]. During calibration and further mobility experiments, the settings were kept the same, for one pass measurement, the analog-to-digital converter (ADC) delay was set to 12 ms, the pushes per bin (V mode) were set to 5, the static T wave height was set to 15 V, the injection time was set to 10 ms and the separation time to 2 ms, finally the eject and acquire wave height was set to 15 V. When two or more passes were used, the resulting ADC delay and separate times were recorded. The obtained data was processed using MassLynx v4.2 & DriftScope v3.0.

3. Results and discussion

3.1. Matrix interferent from poultry liver in LC–MS/MS analysis of stanozolol

A matrix interferent was commonly found during the routine analysis in our laboratory of growth promoter residues in livers using LC–MS/MS, which complicates residue monitoring at low concentration levels. Low-resolution mass spectrometry cannot differentiate or avoid isobaric interferences or separate coeluting isobaric compounds, which are especially frequent when analyzing food samples with complex matrices. To overcome this limitation, highly specific MS/MS detection is used. However, although using LC–MS/MS analysis, some interfering matrix compounds are still observed in the selected MS/MS transitions when analysing e.g. liver tissue samples. Fig. 1 shows that in the matrix of this study, a poultry liver, the interferent elutes at the same retention time as the synthetic anabolic steroid stanozolol and presents a signal for all three monitored transitions (LC–MS/MS operated in multiple reaction monitoring (MRM) mode). Such interference is not only problematic for stanozolol quantification but can even result in false-positive or false-negative results. At present, in the case of stanozolol in liver, its marker metabolite 168-hydroxystanozolol is used in our laboratory for monitoring purposes, partially circumventing the abovementioned problem. However, the ratio of parent compound and metabolite in liver depends on the time between the last administration

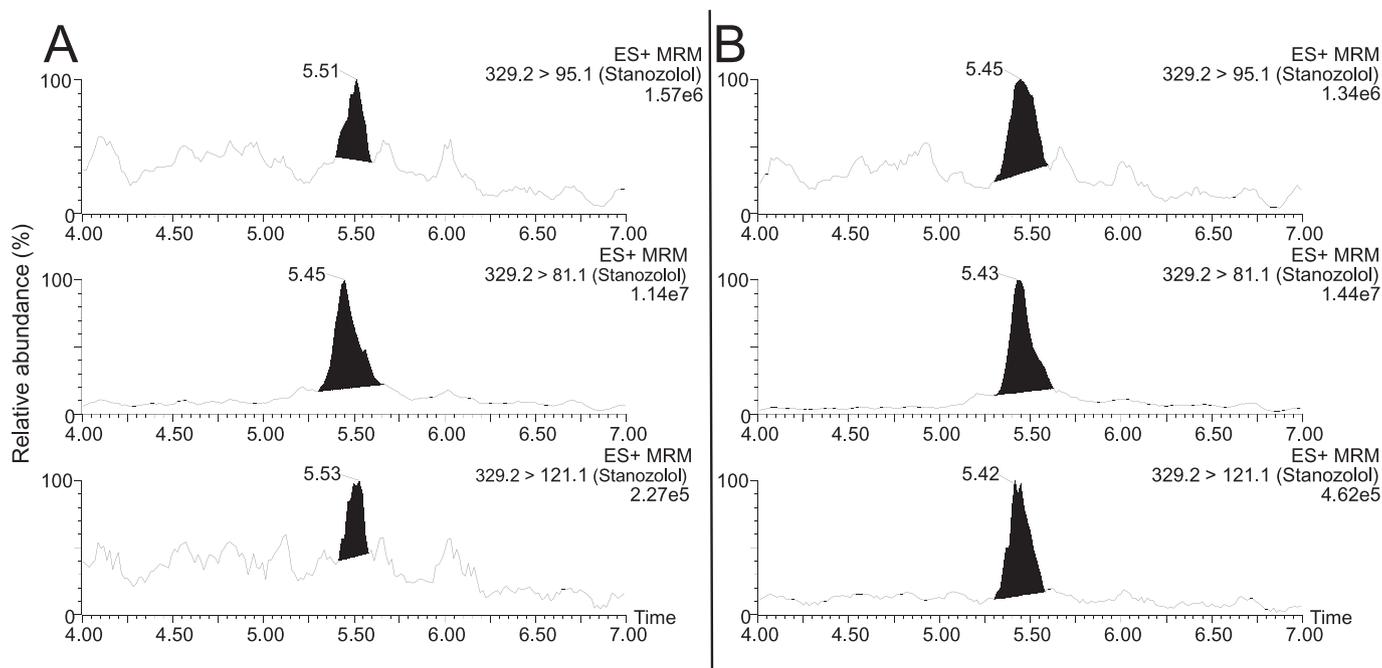


Fig. 1. EIC chromatogram of the LC–MS/MS analysis showing the selected three transitions for the determination of stanozolol on A) a blank liver extract and B) the same liver extract spiked at $1 \mu\text{g kg}^{-1}$ stanozolol.

and slaughter [18].

3.2. Mobility separation of analyte and interfering compound

To overcome the false positive result for stanozolol in blank poultry liver, IMS was tested for separating the interferent from the analyte of interest. First, a standard solution of stanozolol (Fig. 2A), a blank liver sample (Fig. 2B), and a spiked liver extract were injected into the cyclic IMS instrument (LC–IM–TOF–MS) in TOF mode; only to confirm the retention time compared to the routine method (LC–MS/MS). With the EIC window set to 329.2 ± 0.5 Da to simulate the resolution of the LC–MS/MS systems that are used for routine analysis of these steroids in different matrices, the interferent is visible in full scan mode at the same time as stanozolol elutes. As improved mass accuracy and the separation of overlapped isotope cluster ions by HRMS analysis allows for the separation of most isobaric compounds and thereby reduces the number of possible candidates for a given mass-to-charge (m/z), it was found that the interferent could already be separated from stanozolol, i.e. the interferent has an exact mass of 329.0007 Da and that of protonated stanozolol is 329.2587. Thus, a high-resolution instrument was already enough to distinguish the interferent from the analyte of interest in this case. Nevertheless, interferents will not always have a distinct mass that can be easily separated from the analyte, as food control monitoring analyses are often performed on sensitive but low-resolution triple quadrupole LC–MS/MS instruments. Therefore, the poultry liver with stanozolol was further analysed by ion mobility as an unresolved case. Additionally, upon fragmentation of the interferent in the transfer region of the LC–IM–TOF–MS instrument (30 eV), the main fragment ion has the same exact mass as the main fragment from stanozolol (m/z 80.9721). However, the overall intensity was too low for further fragmentation or mobility experiments (data not shown).

To study the performance of ion mobility in this case, the spiked extract was injected using the ion-mobility separation possibilities of the cyclic IMS instrument. As can be observed in Fig. 3, after one pass only, the drift time values of stanozolol (30.8 ms, Fig. 3A) and the interferent (24.9 ms, Fig. 3B) were different. Thus, stanozolol and the interfering peak were mobility-separated without needing multiple passes. Although they have almost identical masses, such a big difference in

drift time indicates that the shape of the matrix molecule is entirely different from that of stanozolol. Additionally, and as a final check, DriftScope software was subsequently used to extract the MS spectra of the mobility-separated species and revealed the same exact mass values of the IM separated stanozolol and interferent peak as those found by HRMS analysis (Fig. 2). Thus eventually confirming the mobility separation of the interfering compound and stanozolol.

3.3. CCS for additional identification

To determine the CCS values of the targeted compound, a logarithmic CCS calibration curve was constructed ($R^2 = 0.997$) (see section 2.5). The drift time of stanozolol ($[M + H]^+$) for 1, 2 and 3 passes were determined by direct infusion of the target compound, and the calibration curve together with the recorded ADC delay was used to calculate the corresponding CCS values (Table 1). Increasing the number of passes results in a higher mobility resolution, which might be needed for the separation of interferents. However, each additional pass also results in the loss of a percentage of the available ions, resulting in a slightly lower sensitivity [14]. The obtained CCS values for the recorded three passes had similar values (with a relative standard deviation of only 0.94 %). Additionally, the obtained experimental CCS values for stanozolol $[M + H]^+$ were compared with those observed in the literature by Hernández-Mesa et al. [19], who also used traveling wave ion mobility (TWIMS). The authors presented a CCS value of protonated stanozolol of 190.3 \AA^2 . As can be observed in Table 1, the experimentally determined CCS values and the reference value are in line, within a deviation window < 2 % for all three passes.

Next, the CCS calibration curve was used to analyze the liver sample and determine the CCS values of stanozolol and the interferent from their drift times calculated from the LC–IM–MS analysis, see Table 1. Again, the CCS value found for stanozolol in the sample 189.9 \AA^2 closely matches the value reported in the literature (0.23 % difference). Moreover, the CCS value of the interferent 161.7 \AA^2 is clearly different from the value found for stanozolol (16.2 % difference). Therefore, having access to an additional characteristic of a given compound, i.e. the CCS, is a clear benefit when analyzing residues in complex matrices. Although the interferent was (in this case) already separated after a

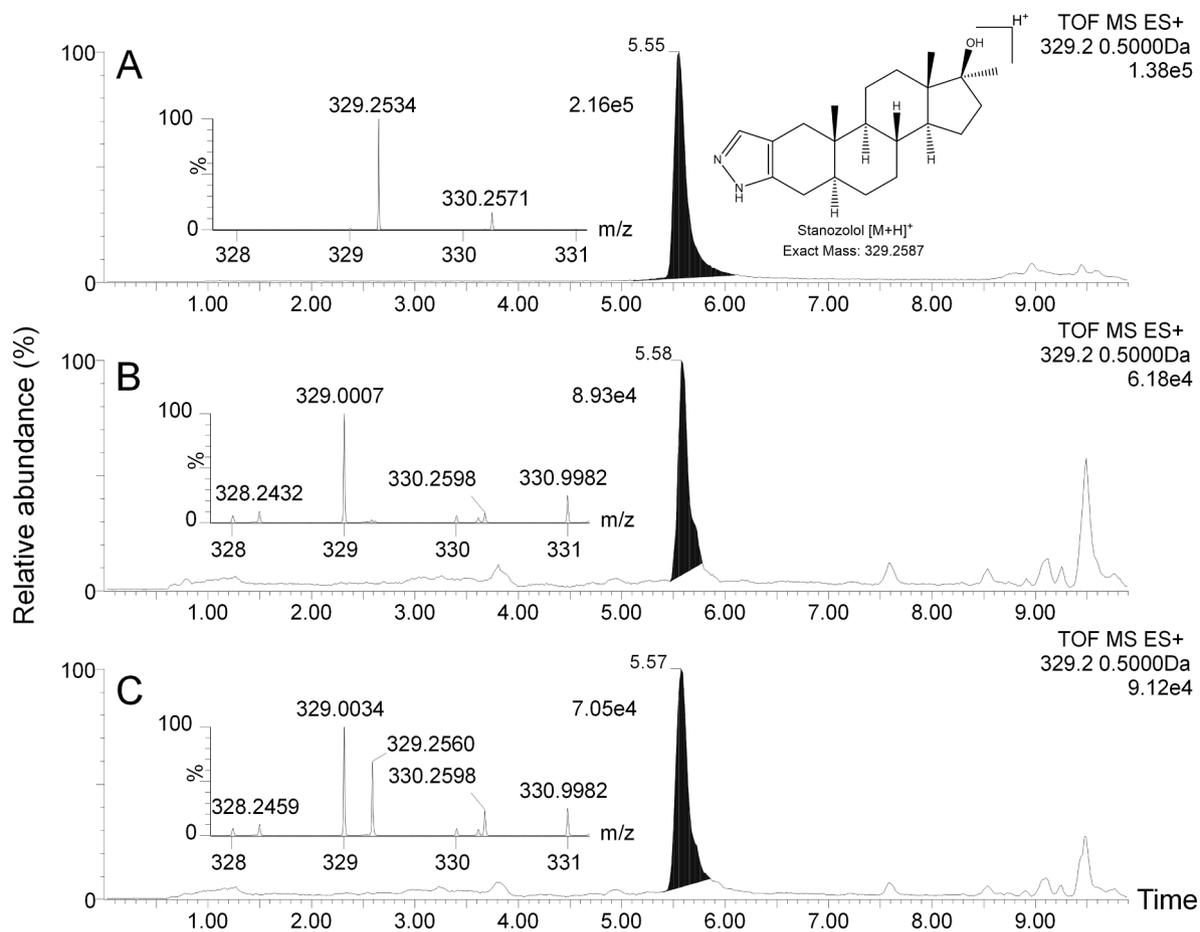


Fig. 2. LC-TOF-MS EIC chromatograms of A) stanozolol standard solution, B) blank liver extract, C) spiked liver extract. Insert MS spectra show averaged spectra of peak at ± 5.6 min.

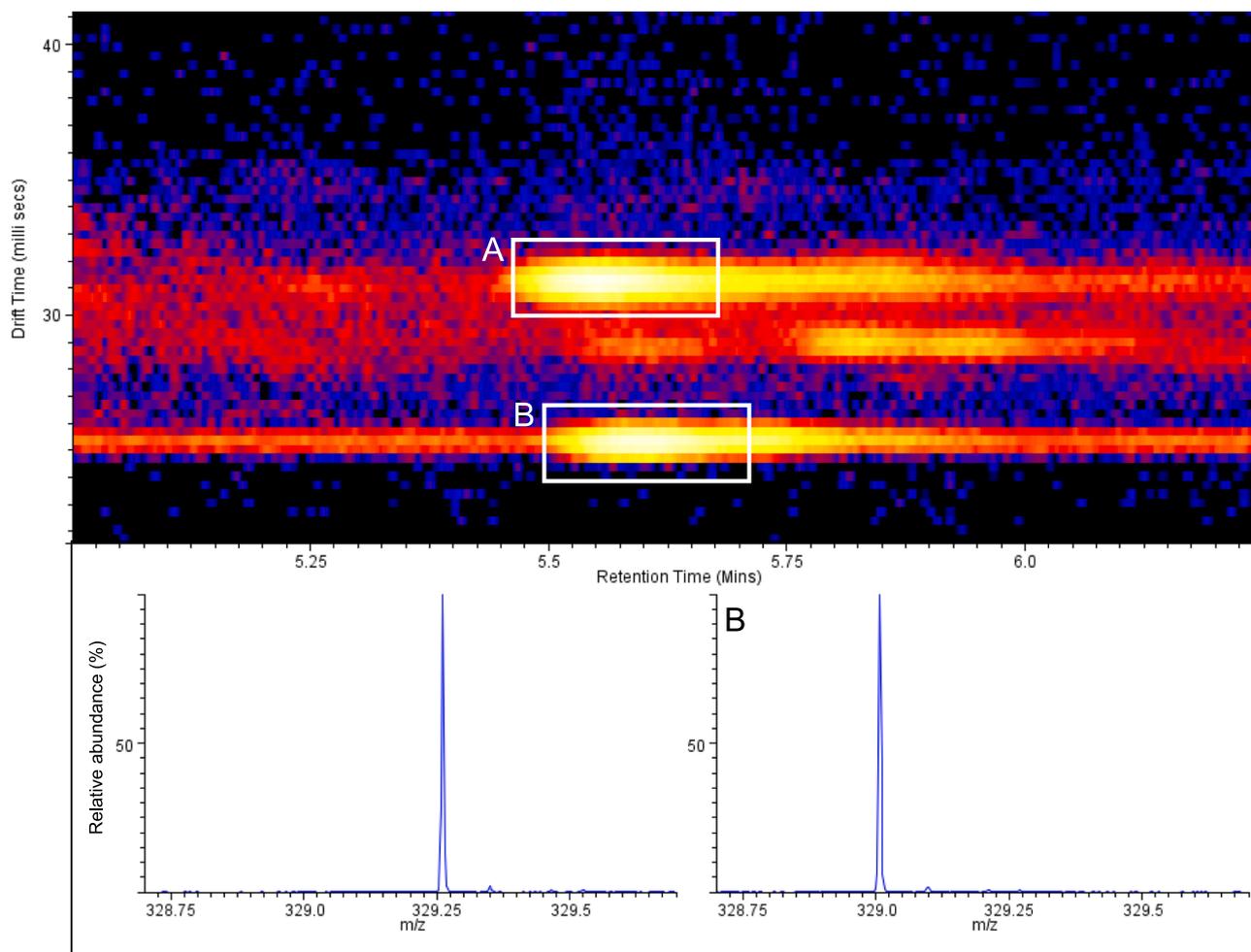


Fig. 3. Upper panel: 3D drift scope plot using an EIC window of 329.2 ± 0.5 Da to simulate low resolution instrument. Lower panel: High resolution MS spectra from selected areas in the 3D plot, A: stanozolol m/z 329.2587, B: interferent m/z 329.0007.

Table 1

Experimentally determined CCS values of stanozolol and interferent for different cycles and comparison (in % Difference) to the CCS value for stanozolol from literature (190.3 \AA^2) [19].

Experiment	Cycles	Drift time (ms)	CCS (\AA^2)	% Difference
Stanozolol, infusion	1	30.8	190.2	0.05
Stanozolol, infusion	2	51.2	193.8	1.83
Stanozolol, infusion	3	69.3	191.8	0.80
Stanozolol, LC-IM-MS	1	30.8	189.9	0.23
Interferent, LC-IM-MS	1	24.9	161.7	16.2

single pass on this advanced ion mobility platform, it suggests that less advanced, more accessible ion mobility platforms combined with routinely used triple quadrupole LC-MS/MS platforms might already be able to tackle the problems observed in routine residue analysis of complex matrices.

4. Conclusions

MS analysis of complex food samples for enforcement purposes has always been challenged by interference from matrix compounds. Extensive clean-up methods, often based on solid phase extraction, combined with separation techniques before the instrumental MS analysis are needed. These comprise, e.g. of HPLC, UPLC and LCxLC systems [20]. LC-IM-MS is not yet routinely used to remove interferences, which separates compounds based on their size and shape. The present study investigated an interfering signal for stanozolol commonly

found in routine LC-MS/MS analysis of liver samples using a newly available cIMS platform. Although applying a high-resolution mass spectrometry system already enabled to differentiate the target compound from the interfering peak by exact mass in this case, this might not always be feasible for other routine food analyses suffering from interfering matrix compounds. The newly developed LC-IM-MS method in this study allowed for an additional differentiation between the target compound and the interfering compound by determination of the CCS values. Peak separation was already achieved after a single pass, showing the potential of IMS as an additional separation dimension. Additionally, the calculated CCS values of the target compound at three different passes were very similar to those previously reported by others. They were clearly different from the CCS value of the interfering compound. This demonstrates that, besides the retention time and m/z value, an analyte's CCS value can be used as a valuable extra identification characteristic for a given compound, especially when interfering signals make identification using the conventional identification points impossible. It thus highlights ion mobility's potential as an additional separation dimension for food control monitoring analyses.

CRedit authorship contribution statement

Sjors Rasker: Writing – original draft, Methodology, Investigation, Conceptualization. **Marco H. Blokland:** Writing – review & editing, Conceptualization. **Toine F.H. Bovee:** Writing – review & editing, Project administration, Funding acquisition. **Ane Arrizabalaga-Larranaga:** Writing – original draft, Supervision, Project administration,

Methodology, Investigation, 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.

Data availability

Data will be made available on request.

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References

- [1] European Commission. (2017). 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. *Official Journal of the European Union*(L 95/1).
- [2] S. Aslantas, O. Golge, M. González-Curbelo, B. Kabak, Determination of 355 pesticides in lemon and lemon juice by LC-MS/MS and GC-MS/MS, *Foods* 12 (9) (2023), <https://doi.org/10.3390/foods12091812>.
- [3] J. Wang, W. Zhao, W. Guo, Y. Li, R. Jiang, H. Li, S. Wang, Z. Li, Simultaneous screening and analysis of 155 veterinary drugs in livestock foods using ultra-high performance liquid chromatography tandem quadrupole linear-ion-trap mass spectrometry, *Food Chem.* 393 (2022) 133260, <https://doi.org/10.1016/j.foodchem.2022.133260>.
- [4] Y. Zhu, X. Jiang, D. Shen, J. Mao, Y. Cao, K. Zhang, J. Peng, F. Dong, N. Wang, K. He, A one-step solid-phase extraction with UHPLC-MS/MS for fast and accurate determination of multi-class veterinary drugs in animal muscles, *Food Chem.* 428 (2023) 136712, <https://doi.org/10.1016/j.foodchem.2023.136712>.
- [5] E. Jongedijk, M. Fifeik, A. Arrizabalaga-Larrañaga, J. Polzer, M. Blokland, S. Sterk, Use of high-resolution mass spectrometry for veterinary drug multi-residue analysis, *Food Control* 145 (2023) 109488, <https://doi.org/10.1016/j.foodcont.2022.109488>.
- [6] D. Steiner, A. Malachová, M. Sulyok, R. Krska, Challenges and future directions in LC-MS-based multiclass method development for the quantification of food contaminants, *Anal Bioanal Chem* 413 (1) (2021) 25–34, <https://doi.org/10.1007/s00216-020-03015-7>.
- [7] S. Uclés, A. Lozano, A. Sosa, P. Parrilla Vázquez, A. Valverde, A.R. Fernández-Alba, Matrix interference evaluation employing GC and LC coupled to triple quadrupole tandem mass spectrometry, *Talanta* 174 (2017) 72–81, <https://doi.org/10.1016/j.talanta.2017.05.068>.
- [8] M. Hernández-Mesa, D. Ropartz, A.M. García-Campana, H. Rogniaux, G. Dervilly-Pinel, B. Le Bizec, Ion mobility spectrometry in food analysis: principles, Current Applications and Future Trends. *Molecules* 24 (15) (2019) 2706. <https://www.mdpi.com/1420-3049/24/15/2706>.
- [9] E. te Brinke, A. Arrizabalaga-Larrañaga, M.H. Blokland, Insights of ion mobility spectrometry and its application on food safety and authenticity: a review, *Anal. Chim. Acta* 1222 (2022) 340039, <https://doi.org/10.1016/j.aca.2022.340039>.
- [10] C. Laphorn, F. Pullen, B.Z. Chowdhry, Ion mobility spectrometry-mass spectrometry (IMS-MS) of small molecules: separating and assigning structures to ions, *Mass Spectrom Rev* 32 (1) (2013) 43–71, <https://doi.org/10.1002/mas.21349>.
- [11] European Commission.COMMISSION IMPLEMENTING REGULATION (EU), 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 Official Journal of the European Union(L 180/84) 2021/808 of 22 March 2021.
- [12] M.L. Feuerstein, M. Hernández-Mesa, A. Kiehne, B. Le Bizec, S. Hann, G. Dervilly, T. Causon, Comparability of steroid collision cross sections using three different IM-HRMS technologies: an interplatform study, *J. Am. Soc. Mass Spectrom.* 33 (10) (2022) 1951–1959, <https://doi.org/10.1021/jasms.2c00196>.
- [13] M. Hernández-Mesa, G. Dervilly, B. Le Bizec, Traveling wave ion mobility-mass spectrometry to address chemical food safety issues, in: H. Koolen (Ed.), *Mass Spectrometry for Food Analysis*, Springer, US, 2022, pp. 259–280, https://doi.org/10.1007/978-1-0716-2107-3_20.
- [14] K. Giles, J. Ujma, J. Wildgoose, S. Pringle, K. Richardson, D. Langridge, M. Green, A cyclic ion mobility-mass spectrometry system, *Anal. Chem.* 91 (13) (2019) 8564–8573, <https://doi.org/10.1021/acs.analchem.9b01838>.
- [15] 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 (8) (2018) 2206–2217, <https://doi.org/10.1007/s12161-018-1164-7>.
- [16] M. McCullagh, S. Gosciny, M. Palmer, J. Ujma, Investigations into pesticide charge site isomers using conventional IM and cIM systems, *Talanta* 234 (2021) 122604, <https://doi.org/10.1016/j.talanta.2021.122604>.
- [17] M. McCullagh, M. Palmer, A. Muck, S. Gosciny, J. Ujma, Determination of the relative CCS of pesticide promomers and conformers using linear and multi-pass cyclic TWIM ion mobility spectrometry [poster]. 69th ASMS Conference on Mass Spectrometry and Allied Topics, 2021.
- [18] S. Poelmans, K. De Wasch, H.F. De Brabander, M. Van De Wiele, D. Courtheyn, L. A. van Ginkel, S.S. Sterk, P. Delahaut, M. Dubois, R. Schilt, M. Nielen, J. Vercammen, S. Impens, R. Stephany, T. Hamoir, G. Pottie, C. Van Poucke, C. Van Peteghem, Analytical possibilities for the detection of stanzolol and its metabolites, *Anal. Chim. Acta* 473 (1) (2002) 39–47, [https://doi.org/10.1016/S0003-2670\(02\)00672-4](https://doi.org/10.1016/S0003-2670(02)00672-4).
- [19] M. Hernández-Mesa, B. Le Bizec, F. Monteau, A.M. García-Campana, G. Dervilly-Pinel, Collision cross section (CCS) database: an additional measure to Characterize steroids, *Anal. Chem.* 90 (7) (2018) 4616–4625, <https://doi.org/10.1021/acs.analchem.7b05117>.
- [20] M.H. Blokland, P.W. Zootjes, L.A. Van Ginkel, M.G.M. Van De Schans, S.S. Sterk, T.F.H. Bovee, Multiclass screening in urine by comprehensive two-dimensional liquid chromatography time of flight mass spectrometry for residues of sulphonamides, beta-agonists and steroids, *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 35 (9) (2018) 1703–1715, <https://doi.org/10.1080/19440049.2018.1506160>.