# Bovine Teeth: A new Forensic Matrix for the detection of Regulated and Banned Substances

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## Background and Objectives

To investigate illicit administration of regulated and banned substances used for growth-promoting purposes in cattle breeding, it is common to analyse the usual matrices like urine, feed, animal tissue, and hair samples nowadays.

We wondered if there is a different type of sample material available that could overcome these disadvantages? A requirement is that the samples should be traceable to the animals and be easy to collect, for example, at slaughterhouses. Possible 'offal'?

The question arised if bovine teeth could be the **'new'** matrix. Therefore, we set to develop and validate a liquid chromatography tandem-mass spectrometry (UHPLC-MS/MS) method to detect different class of compounds (beta-agonists, NSAID's and a selection of veterinary drugs (tetracyclines, quinolines, macrolides and sulfonamides) in bovine teeth.

## General pre-treatment Bovine teeth
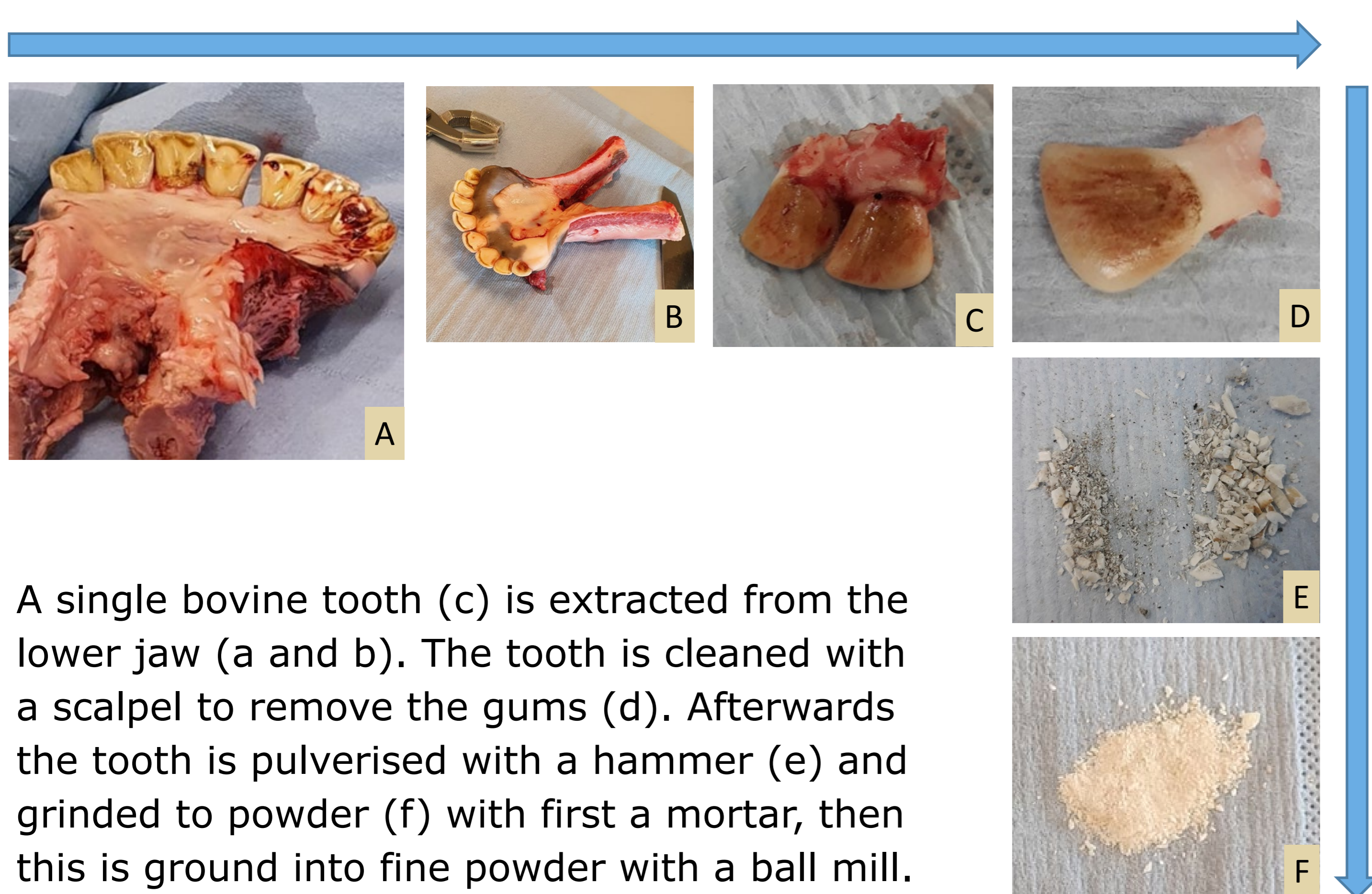


A single bovine tooth (c) is extracted from the lower jaw (a and b). The tooth is cleaned with a scalpel to remove the gums (d). Afterwards the tooth is pulverised with a hammer (e) and grinded to powder (f) with first a mortar, then this is ground into fine powder with a ball mill.

## Analyis of Bovine Teeth

At the fine teeth powder extraction solvent (solvent depends on the class of compounds) is added. After centrifugation for the antibiotics and the beta-agonists a dedicated Solid Phase Extraction is applied for further purification and for the NSAID's Bondesil PSA (40 µm) is added for further purification. After these clean-up the extracts are analysed with UHPLC-MS/MS.
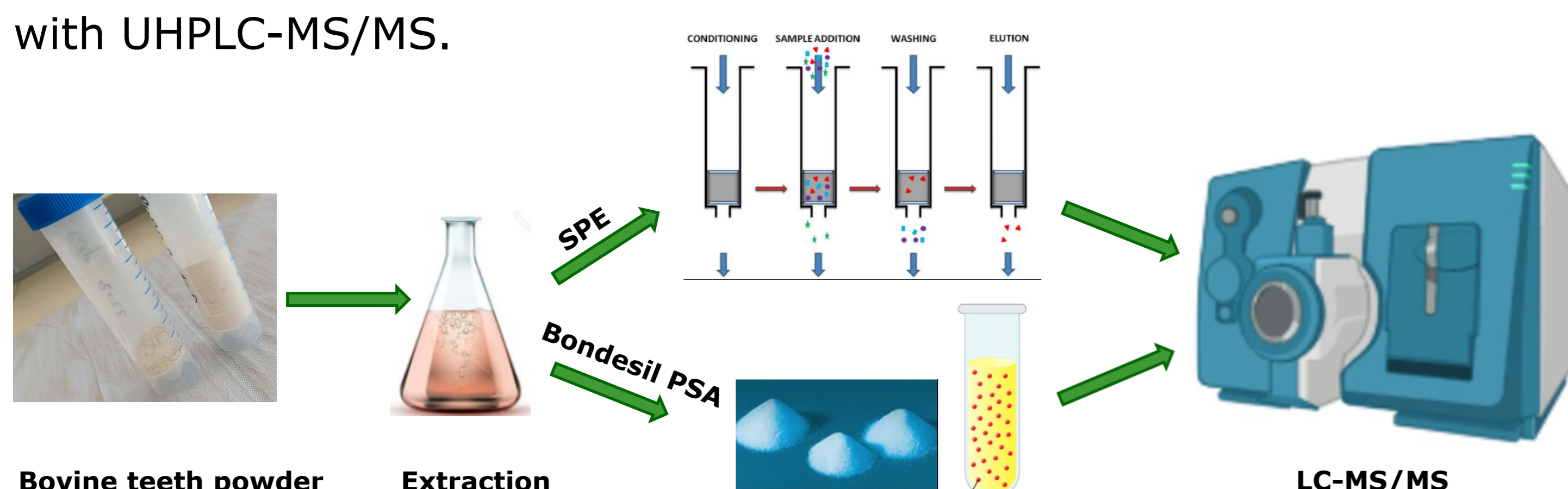


## Results validation

All three the methods are fully validated according the Commision Implementing Regulation (EU) 2021/808.
In the table below some of the validation parameters are summarised.

| Compound class (number of compounds) | CCα (range, µg/kg) | Precision (range, n=21, %) | Intra lab reproducability (range, n=21, %) |
| --- | --- | --- | --- |
| Tetracyclines (4) | 7.4 – 20* | 96 – 110** | 4.2 – 20** |
| Quinolones (10) | 3.2 – 26.3 | 91 – 110 | 2.3 – 21 |
| Macrolides (11) | 4.4 – 93 | 88 – 108** | 2.4 – 25** |
| Sulfonamides (18) | 3.3 - 14 | 95 - 104 | 2.0 – 9.8 |
| NSAID's (14) | 6.3 – 62 | 92 - 119 | 2.4 – 19.2 |
| Beta-agonists (26) | 0.22 – 50 | 83 - 108 | 1.9 – 23*** |

\* CCα for oxytracycline cannot be determind.
\*\* Six compounds (oxytetracline, neospiramycine, pirlimycine, tildipyrosine, tulathromycine and tilmicosine) did not meet the criteria. These validation results are <u>not included in</u> the table.
\*\* Procaterol and tulobuterol did not meet the criteria, these validation results are <u>not inluced</u> in the tabel.

## Results samples collected, real cases

<u>Case 1</u>. From a cadaver of a bovine calf, which was suspected for an illicit adminstration of flumequine, the jaw was sampled and the extracted teeth were analysed with the antibiotic method for the presence of flumequine. In these teeth also oxytetracycline and doxycycline was detected.
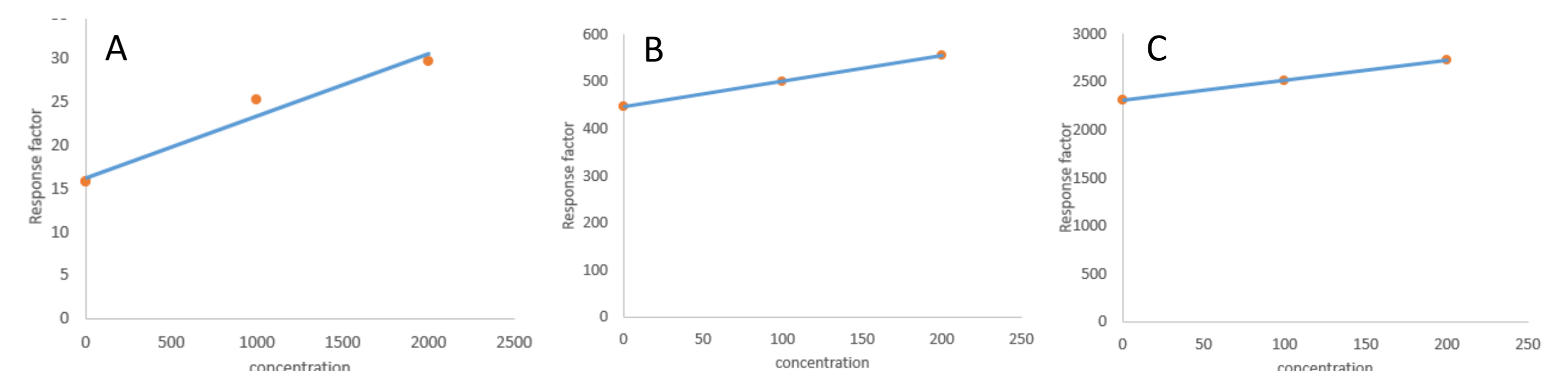


**Figure 1.** Multi Level Standard Addition analyses of flumequine (A), oxytetracycline (B) and doxycycline (C) in bovine teeth. Flumequine was detected with a concentration of 2200 µg/kg, oxytetracycline was detected with a concentration of 800 µg/kg and doxycycline was detected with a concentration of 1100 µg/kg.

<u>Case 2</u>. Teeth were collected from a treated calve (Paracetamol animal trial) and analysed with the NSAID method for the presence of paracetamol.
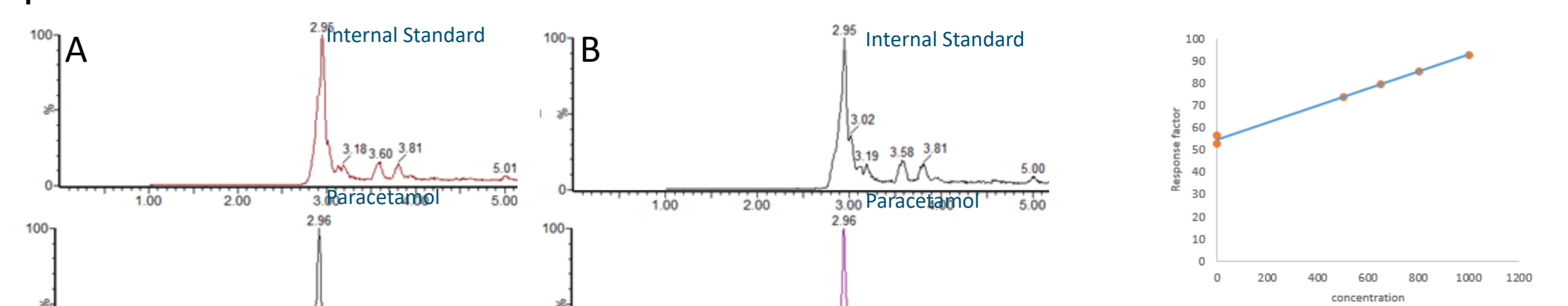


**Figure 2.** Chromatogram of teeth from the treated calve (A) and teeth from the treated calve with an addition of 1250 µg/kg paracetamol (B).

## Conclusion

From the validation results and the findings of the administered compounds in the bovine teeth samples from real cases, it proves that the developed method is fit for the purpose and that bovine teeth are an excellent matrix to detect illicit administration of veterinary drugs.

## Acknowledgements