# Application Note

## Rapid validation of an LC-MS approach to differentiate tainted and untainted boar meat based on compound biomarkers

## Overview:

- **A broad nontargeted metabolomics study** was performed to establish the feasibility of an LC-MS/MS approach to differentiate samples from boars with tainted meat from those without taint.[1]
- **Progenesis CoMet** reanalysed113 LC-MS runs from this study to determine if we could provide the same proof of concept within a few hours compared to several weeks.
- **Data analysis took <3 hours** including searches against an HMDB compound database[2] to generate a sub-set of 18 compounds with significant abundances differences between the two groups. **10/18 compounds** were found to be **common to the original study results**, three with identifications confirmed.
- **Progenesis CoMet showed advantages in speeding up data analysis for discovery-focussed experiments** prior to committing further resource into method development and targeted metabolomics.

## Introduction & Method:

In many countries male piglets are castrated shortly after birth to avoid the production of meat with an unpleasant smell and flavor known as boar taint. Extensive research has been carried out during the last 40 years to delineate compounds that are responsible for this problem. The most frequently candidates are androstenone, skatole and indole[1]. However, the level of these compounds does not always correlate with results from classical sensory panels and other factors are thought to be involved. Research by *Olson et al*[1] demonstrated the feasibility of LC-MS/MS to discriminate tainted and non-tainted carcasses based on a sub-set of biomarker compounds. This provides potential for a quantitative metabolomics assay to be applied early in production that can avoid costumer complaints, reduce the need for castration and provide better efficiency in stock rearing[1].

A set of113 LC-MS runs generated by the original research comprising 3 technical replicates of adipose tissue extracts from 32 individual pigs, (16 qualified as "untainted" and 17 qualified as "tainted" by a sensory panel), controls (a pool of adipose tissue extract from all 32 pigs) and extraction process blanks were loaded into Progenesis CoMet. The simple, automatic workflow took <3 hours to retention-time align all runs against a pool sample run, peak pick, deconvolute adduct ions and quantify ion abundance of 2,532 compounds. All detected compounds were searched against the HDMB SDF database v3.0 using our unique search tool, MetaScope. 1,127 compound identifications were returned and automatically linked to the quantified compounds. Three separate experiment designs were set up within the same analysed experiment, each comparing a set of technical replicates. The significantly different ($p<0.01$) compounds common to all groups of technical replicates were selected using the "tagging" feature. PCA plots were made using the sub-set of tagged compounds and the identifications of these 18 significant compounds were exported and compared to the original study results.

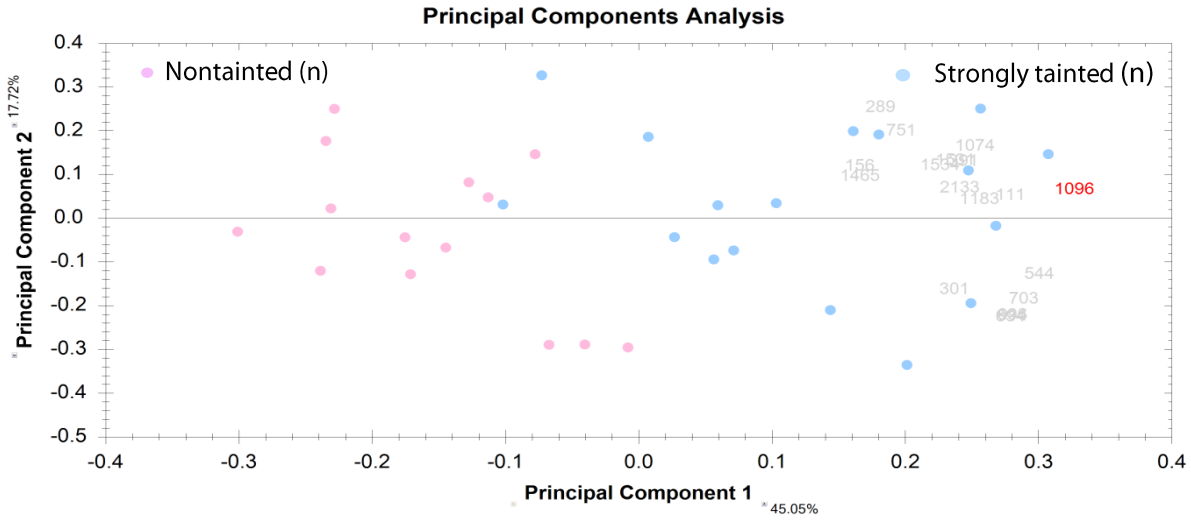


**Figure 1:** Principle Components Analysis (PCA) following analysis by Progenesis CoMet showing separation between samples taken from 32 individual pigs, 17 identified as "strongly tainted" (**blue dots**) and 16 identified as "non-tainted" (**pink dots**) by a sensory panel. The PCA plot was generated using a sub-set of compounds that were significantly different between groups, p-value <0.01, and common to all three sets of technical replicates. This list of 18 significant compounds included 10 compounds or compound ions common to the original research.

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# Results and Conclusion

Our re-analysis took a much simpler approach, which did not directly replicate the approaches used for identification searches or selection of significant compounds based on relative quantification compared. Specifically we just took automatically generated results from Progenesis CoMet with no optimisation of analysis parameters to generate a list of "significant" compounds and a PCA plot. We were also limited to searching a different database (HMDB). Despite this, the final list of 18 compounds generated by our analysis included testosterone, androstenadione and 3-oxohexadeanoic acid that were identified in the orginal study[1]. We also found another 7 compounds common to the original study without identifications. The PCA plot generated from our data analysis (see above) also showed that we could discriminate tainted from untainated samples. These results agree with the original study in that they demonstrate feasibility of the LC-MS approach to identify tainted sample from untainted samples and provide a basis for further study but here we managed simpler, faster data-analysis to achieve the same end results.

| m/z | RT (min) | Anova p-value | Fold change | Putative identifications |
|---|---|---|---|---|
| **289.2167** | **7.83** | **$4.22 \times 10^{-14}$** | **30** | **Testosterone** |
| **287.2012** | **8.88** | **$2.35 \times 10^{-5}$** | **24** | **Androstanedione** |
| 148.0762 | 6.08 | $5.38 \times 10^{-4}$ | 6 | Normetanephrine, Methylnoradrenaline, Epinephrine, Norsalsolinol, 3-Pyridinebutanoic acid, Benzocaine, L-Phenylalanine, Indole-3-carbinol, Benzaldehyde |
| 192.0242 | 5.38 | 0.001 | 2 | DL-O-Phosphoserine, Phosphoserine, L-4-Hydroxyglutamate semialdehyde, D-Glutamic acid, L-Glutamic acid, N-Methyl-D-aspartic acid, N-Acetylserine, O-Acetylserine |
| 246.1704 | 5.54 | 0.002 | 1.6 | Hydroxyvalerylcarnitine, Isovalerylcarnitine, 2-Methylbutyroylcarnitine, Valerylcarnitine |
| 496.3402 | 11.39 | 0.003 | 1.5 | LysoPC(16:0), Docosa-4,7,10,13,16-pentaenoyl carnitine, Clupanodonyl carnitine |
| 217.1956 | 11.79 | 0.004 | 1.5 | 7,10-Hexadecadienoic acid, 7Z,10Z-Hexadecadienoic acid |
| **235.2059** | **11.79** | **0.001** | **1.5** | **3-Oxohexaecanoic acid** |
| 188.0912 | 5.62 | $7 \times 10^{-4}$ | 6 | 3,4-Dihydroxyphenylglycol |
| 258.1336 | 5.52 | 0.005 | 4 | Glutarylcarnitine |
| 357.2794 | 7.51 | 0.002 | 16 | Tetracosahexaenoic acid |
| **273.2262** | **20.16** | **$8 \times 110^{-4}$** | **present/ absent** | **No indentification** |
| **357.2791** | **7.81** | **$1 \times 10^{-5}$** | **2** | **No indentification** |
| **322.2016** | **5.93** | **0.008** | **4.5** | **No indentification** |
| **261.1447** | **5.52** | **0.002** | **1.5** | **No indentification** |
| **339.0899** | **5.11** | **$6 \times 10^{-4}$** | **1.5** | **No indentification** |
| **386.2534** | **6.4** | **0.007** | **3** | **No indentification** |
| 247.1698 | 5.93 | 0.002 | 3 | No indentification |
| **358.2228** | **5.93** | **0.008** | **6** | **No indentification** |

**Compounds highlighted in green were common between the original study data and re-analysis by Progensis CoMet.**

## Conclusion

Establishing an untargeted approach to metabolomics where there is no set of known answers is a challenge. In this case it is necessary to determine a proof of concept for your chosen sample preparation and data analysis methods. This proof of concept provides the foundation for more extensive method development and validation of putative compound biomarkers.

The orignal study results were generated over several months, requring the use of many different applications, involvement of biostatsticians and signficant resources. LC-MS sample analysis time was also limited. At this stage rapid visualisation of data and confirmation of results would have enabled more confirmatory experiments to be performed while access to essential equipment was limited . Alternatively, the time saved could have enabled more samples to be run or opportunities to refine methods while LC conditions were stable.

In this re-analysis of existing study data we were able to deliver results comparable to those in the original study, which validated an LC-MS/MS method for differentiating samples of "tainted" and "untainted" boar meat based on compound profiles. The data analysis we used to validate the LC-MS approach was performed in hours compared to weeks. The LC-MS method established in this research will be further tested as a more automated and objective way to measure taint in boar meat compared to the current approaches.

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**References:**

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2. Wishart DS, Knox C, Guo AC, et al. HMDB: a knowledgebase for the human metabolome. Nucleic Acids Res. 2009 37(Database issue):D603-610.

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