# TARGETED AND UNTARGETED METABOLOMICS AS AN ENHANCED TOOL FOR THE DETECTION OF JUICE-TO-JUICE ADULTERATION

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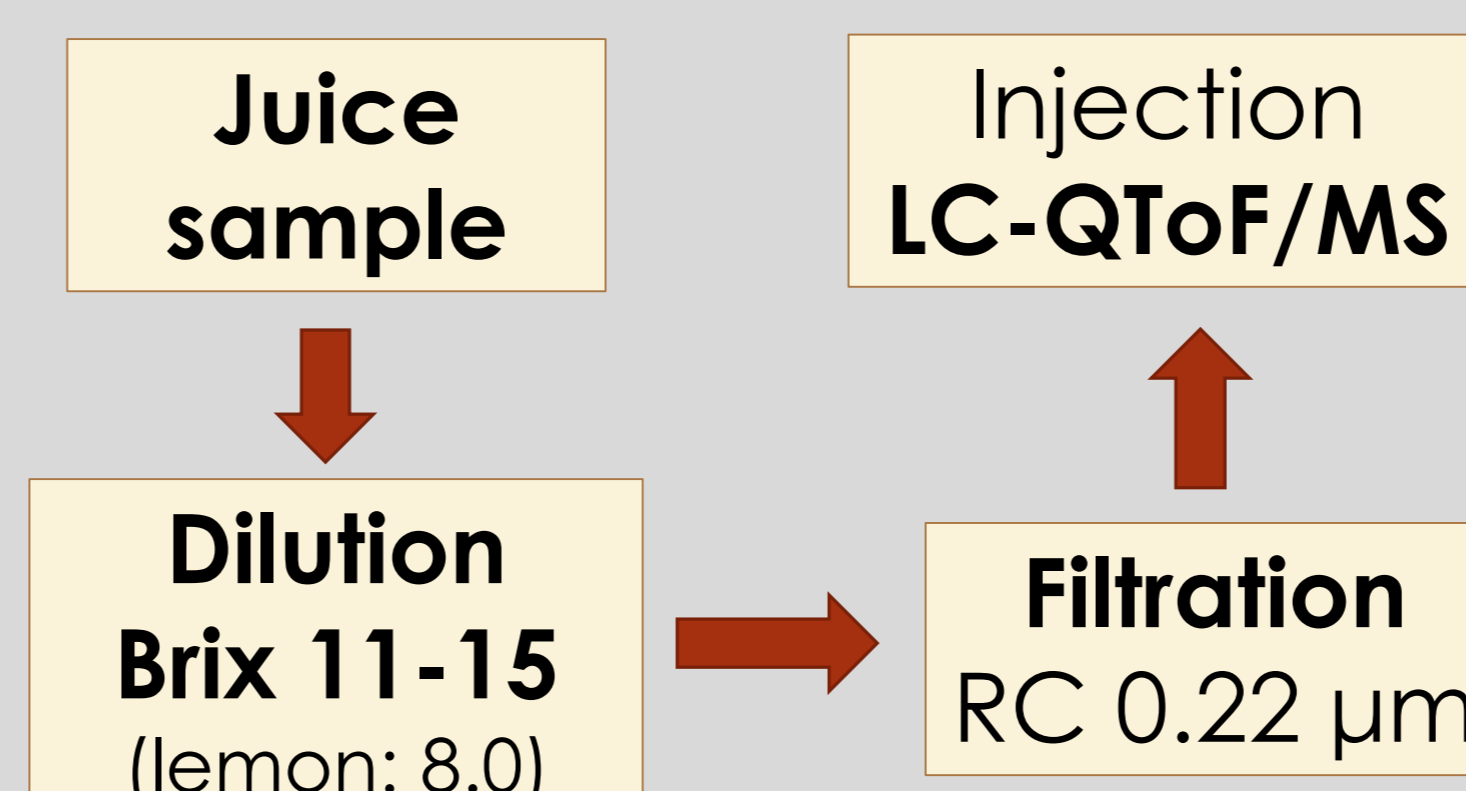
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## INTRODUCTION

Economically motivated adulteration (EMA) of food, also known as food fraud, is the intentional adulteration of food for financial advantage. Fruit juices have been in the top 7 foods reported from 1980 to 2010 as the most common targets for adulteration. Several fruit juices, as **orange** and **pomegranate juice**, have become popular with regard to high levels of antioxidants, presumed to be associated with positive health effects. Similarly to other highly prized food commodities, the economic value and large-scale production of these valuable fruit juices have made them a likely target for adulteration and fraud. One of the most frequent profit-driven fraudulent procedures is extension of authentic juice with cheaper alternatives (typically juices obtained from apples, grapes, grapefruits, etc.) Consequently, there is a substantial need for effective **food control systems** to protect consumers from adulterated food products. In the present study, the main objective was to explore the feasibility of using **targeted** and **untargeted** analysis, using ultra-performance liquid chromatography-quadrupole time of flight mass spectrometry, UPLC-QToF/MS, to discriminate authentic and adulterated fruit juices.

## EXPERIMENTAL

**Instrumentation: UHPLC-QToF/MS**
**Column:** Acclaim C18, 2.1x100 mm, 2.2 µm, (Dionex-Thermo Scientific), **ESI:** Negative (-)
**Gradient elution program:** (A) 5 mM ammonium acetate in $H_2O$/MeOH 90/10, (B) 5 mM ammonium acetate in MeOH
**Acquisition mode:** broad-band Collision Induced Dissociation bbCID, (MS & MS/MS) (Col. Energy MS: 4eV, MS/MS: 25eV)

Bruker Maxis Impact™

Juice sample → Dilution Brix 11-15 (lemon: 8.0) → Filtration RC 0.22 µm → Injection LC-QToF/MS

| Juice | Variety | Origin |
|---|---|---|
| Pomegranate | Hicaz | Turkey |
| Orange | Valencia | Greece |
| Apple | Starkin, Granny, Granny Smith | Greece |
| Lemon | Bertonato, Maglina, Adamopoulou | Greece |
| Red grape | Sangiovese, Montepulcianoo, Lambrusco, Schiava, Shiraz, Ciliegiolo, Merlot | Italy / Puglia |

## RESULTS AND DISCUSSION

### TARGET SCREENING

TargetAnalysis

- Target list included 30 phenolic compounds
- Markers presented linear response in different adulteration percentages

**Identification Criteria**
**deltaRT** ≤ 0.1 min
**Accuracy**: Error ≤ 5 mDa
**Isotopic fit**: ≤ 100 mSigma
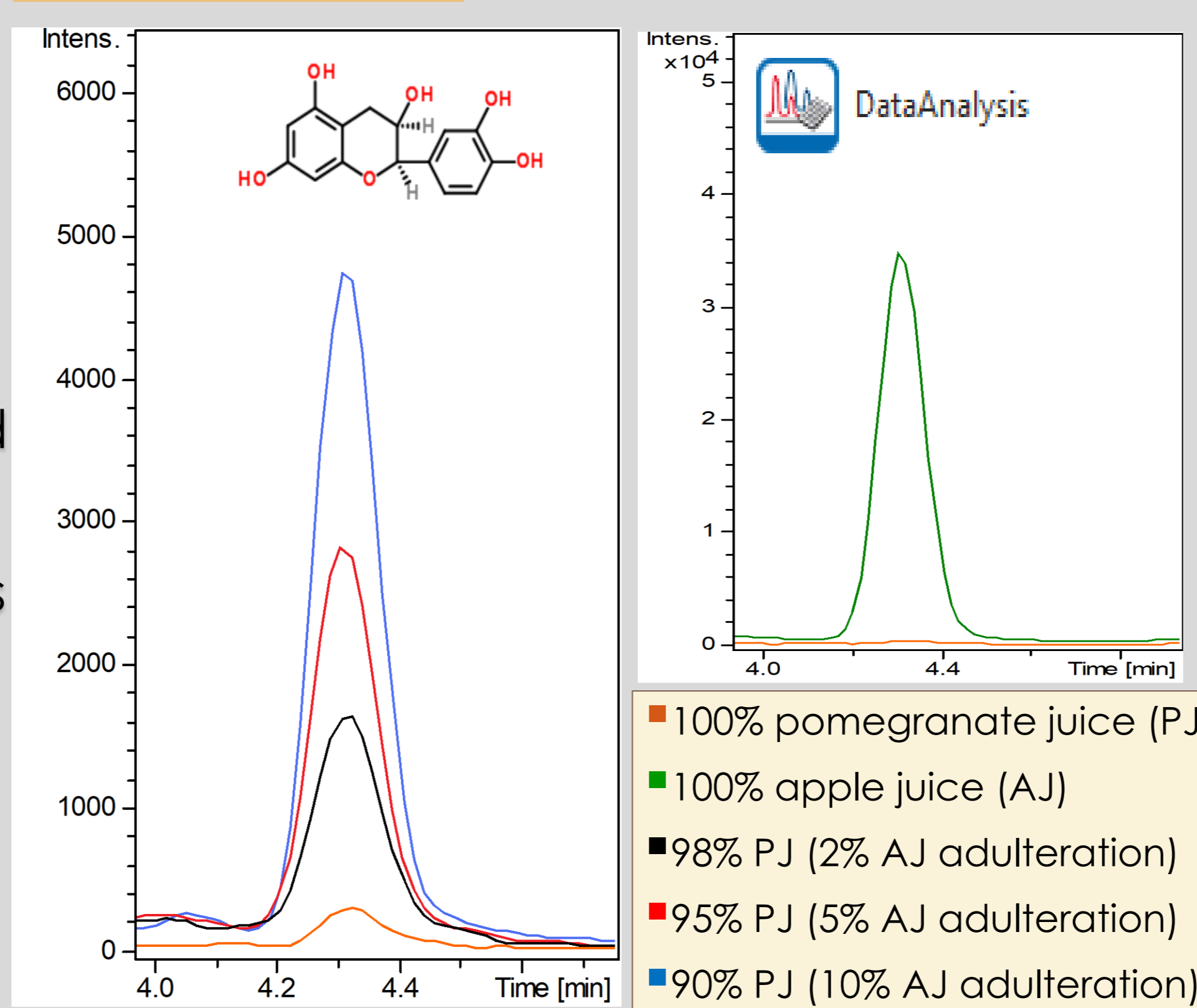**MS/MS fragments**
**Ion Intensity** > 200
**Area** > 800

✓ **Pomegranate and orange juice adulteration was detected down to 2%**

**Epicatechin** was identified as a marker for pomegranate juice adulteration with apple and red grape juice (Fig. 1)

**Hydroxytyrosol** was identified as marker for pomegranate juice adulteration with red and white grape juice

**Eriodictyol** was identified as a marker for orange juice adulteration with lemon (Fig. 2)

**Epicatechin**

DataAnalysis

- 100% pomegranate juice (PJ)
- 100% apple juice (AJ)
- 98% PJ (2% AJ adulteration)
- 95% PJ (5% AJ adulteration)
- 90% PJ (10% AJ adulteration)

**Fig. 1:** XIC of Epicatechin in different ratios of pomegranate juice adulteration with apple juice

**Eriodictyol**

- 100% orange juice (OJ)
- 100% lemon juice (LJ)
- 95% OJ (5% LJ adulteration)
- 90% OJ (10% LJ adulteration)
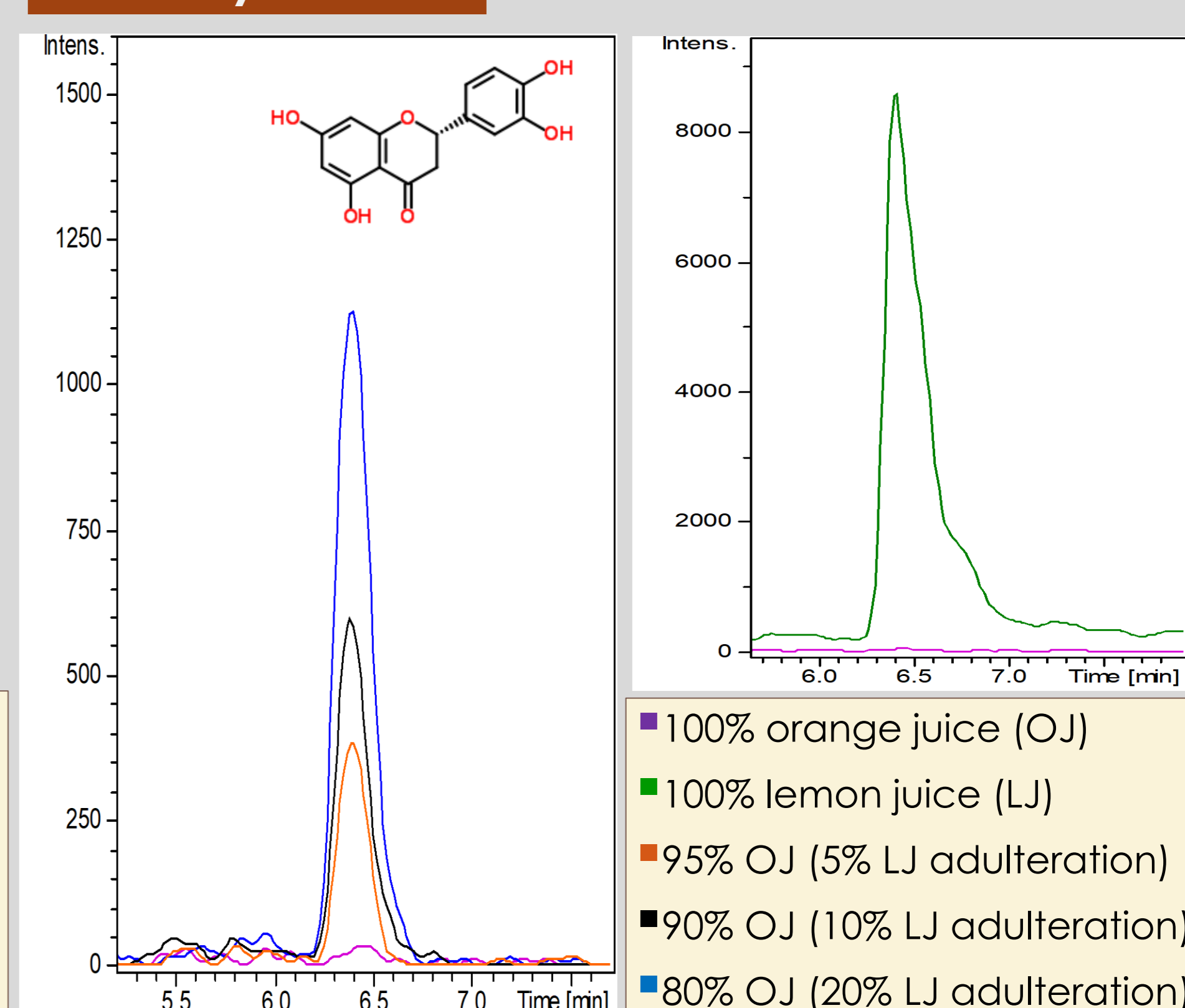- 80% OJ (20% LJ adulteration)

**Fig. 2:** XIC of Eriodictyol in different ratios of orange juice adulteration with lemon juice

### NON-TARGET SCREENING

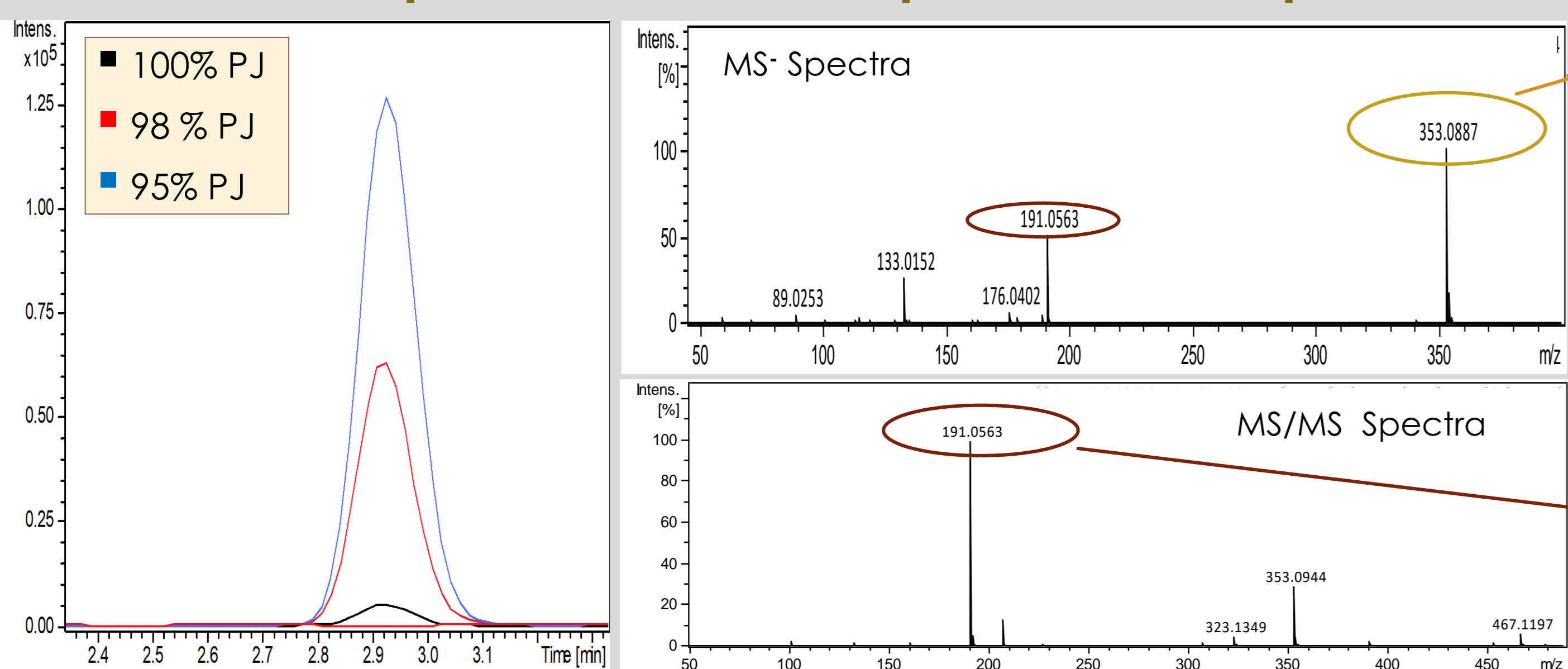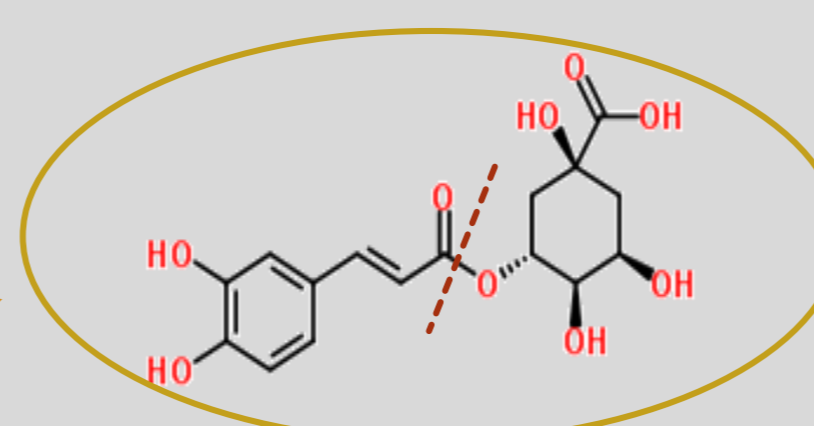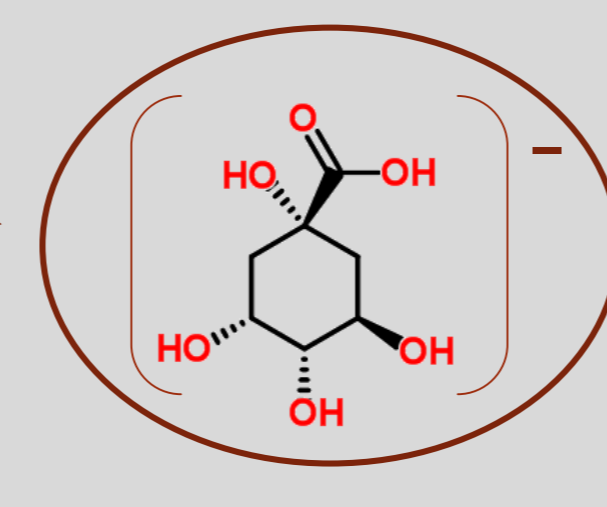**In-house suspect list with 2395 phenolic compounds**

- 100% PJ
- 98 % PJ
- 95% PJ

$MS^-$ Spectra

353.0887
191.0563
133.0152
176.0402
89.0253

MS/MS Spectra

191.0563
353.0944
323.1349
467.1197

**Fig. 3:** XIC, MS and MS/MS spectra of Chlorogenic acid in pomegranate juice samples adulterated with apple juice

**Chlorogenic acid** $C_{16}H_{18}O_9$
Mass accuracy: **0.9 mDa**
Isotopic fit: **5.4 mSigma**

$C_7H_{11}O_6^-$
Mass accuracy: **0.2 mDa**
Isotopic fit: **17.9 mSigma**

✓ **Differential analysis** of pomegranate and apple juice was performed with **Bruker's MetaboliteDetect 2.0.**

✓ Generation of a **peak list of unknowns**

✓ Search for **m/z markers** with a linear response in different adulteration ratios

**Use of in-house QSRR prediction model** [1]

RetTrAMS V 3.0    Metabolite Detect

| Compound | Chlorogenic acid |
|---|---|
| Predicted $t_R$ | 2.7 min |
| Experimental $t_R$ | 2.9 min |
| $\Delta t_R$ | -0.2 min |

1. Differential Analysis (eXpose algorithm, Ratio > 5)
2. Peak Picking (Find Molecular Features algorithm)
3. Screening of m/z of interest

☑ **34 m/z markers were identified to detect 5% adulteration of pomegranate juice with apple juice**

## CONCLUSIONS

❖ Target and non-target screening were used to identify markers indicating pomegranate and orange juice adulteration with apple, grape, lemon and grapefruit juice.
❖ Epicatechin, hydroxytyrosol and eriodictyol were identified at identification confidence level 1 and chlorogenic acid at identification confidence level 2a [2] as markers of the adulteration of authentic pomegranate and orange juices at levels of 10%, 5% and 2% .
❖ Non-target screening revealed 108, 78 and 34 m/z markers indicating 20%, 10% and 5% pomegranate juice adulteration with apple juice, respectively.

## AKNOWLEDGMENTS

**References**
**(1).** Aalizadeh, Thomaidis, Bletsou, Gago-Ferrero (2016) J. Chem. Inf. Model, 56(7), 1384-98. **(2).** Schymanski, Jeon, Gulde, Fenner, Ruff, Singer & Hollender (2014) ES&T, 48(4), 2097-2098.