

Analysis of Organic Acids by Ion Exclusion Chromatography and Single Quadrupole LC/MS

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Introduction

While LC/MS is typically performed in reverse phase mode, organic acids pose a challenge due to their high hydrophilicity and low retention on reverse phase columns. The analysis is further complicated by complex matrices where interfering compounds are often co-eluted.

Organic acid separation by ion exclusion chromatography, also known as ligand exchange chromatography, has been widely adopted as a reliable method of separating organic acids, with UV detection by post-column addition of a pH indicator, such as BTB. However, the detection limits near 10 ppm are often inadequate.

In this study, organic acids were separated by ion exclusion and detected by single quad MS. After confirming sensitivity and linearity, two commercial yoghurt samples was analyzed.

Instrument



Agilent 1260 Infinity LC
Agilent 6120 Single Quad LCMS



Agilent Hi-Plex Column

Experimental

LC

Column : Agilent Hi-Plex H, 300 x 7.7 mm
Mobile Phase A: 0.01% Aqueous Formic Acid
Mobile Phase B: Acetonitrile
A/B: 70/30 (Isocratic)
Flowrate: 0.4 mL/min
Column Temperature: 45 °C
Injection Volume: 10 µL
Analysis time: 22 min

MS

Ion Source: ESI
Gas Flow: N₂, 12 L/min
Gas Temperature: 200 °C
Neblulizer Pressure: 50 psi
Capillary Voltage: 3000 V (negative mode)
MS signal : SIM negative (see table)

SIM table	m/z	fragmentor
Ketoglutaric acid	145	90
Acetic acid	59	120
Pyruvic acid	87	90
Phosphoric acid	97	120
Citric acid	191	110
Malic acid	133	100
Glyoxylic acid	73	110
Ascorbic acid	175	120
Succinic acid	117	100
Lactic acid	89	100
Glycolic acid	75	100
Isovaleric acid	101	100
Valeric acid	101	100

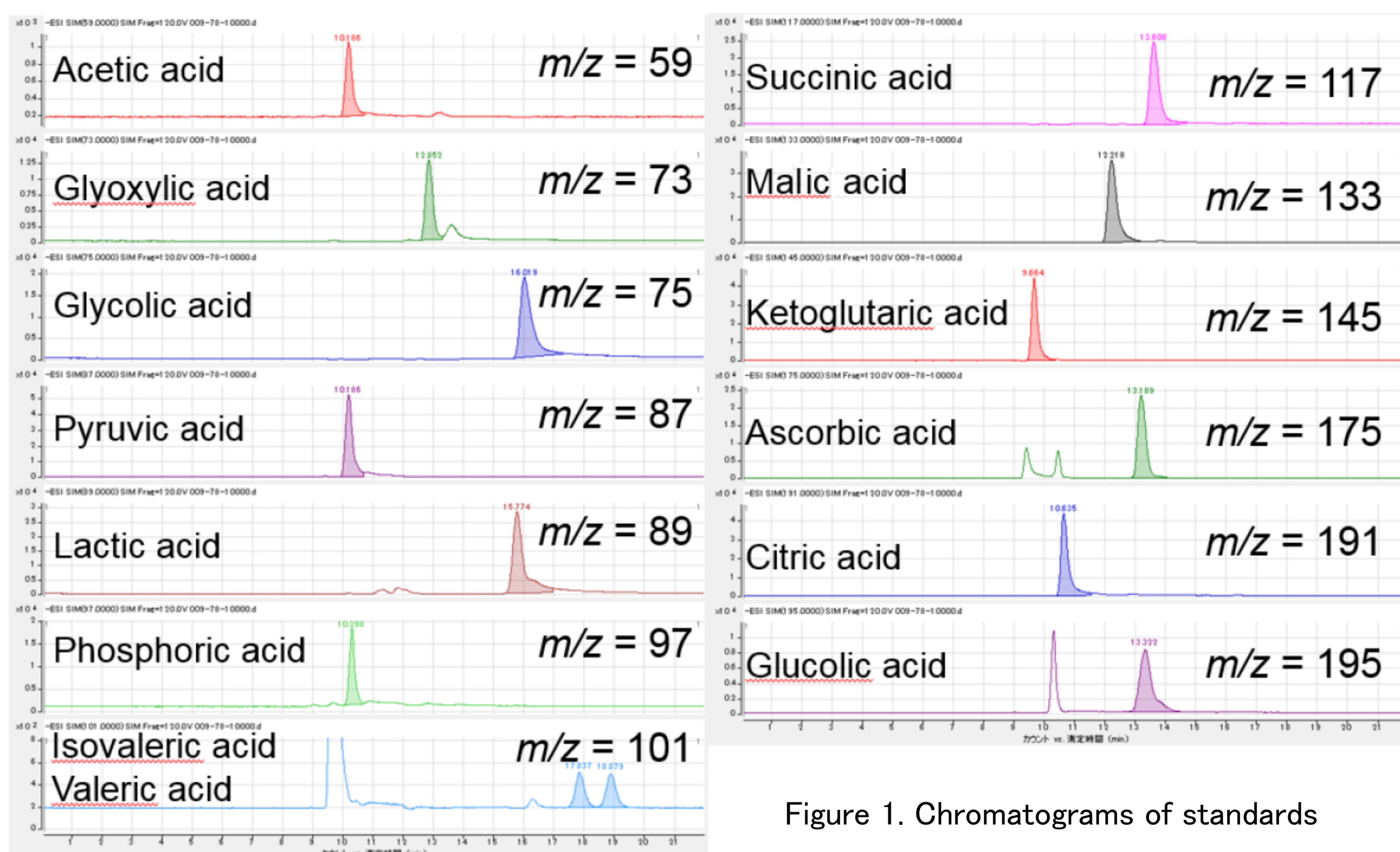


Figure 1. Chromatograms of standards

Results and discussion

Table 2. Peak to peak calculated LOD (S/N=3)

	m/z	LOD(ppb)
acetic acid	59	85.7
glyoxylic acid	73	6.5
glycolic acid	75	4.5
pyruvic acid	87	2.5
lactic acid	89	2.6
phosphoric acid	97	263.2
isovaleric acid	101	483.9
valeric acid	101	681.8
succinic acid	117	8.1
malic acid	133	2.8
ketoglutaric acid	145	3.1
ascorbic acid	175	4.9
citric acid	191	31.9
glucolic acid	195	5.7

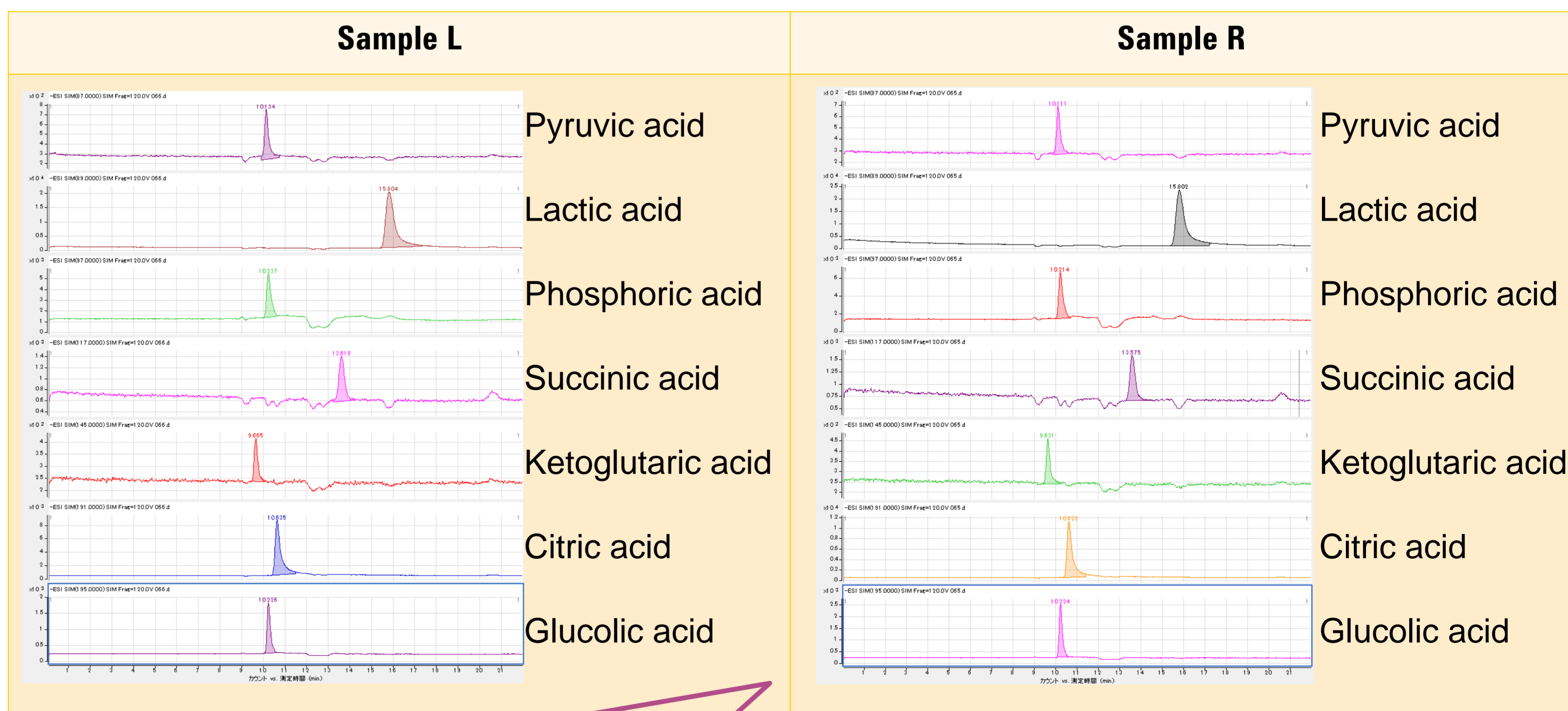


Figure 3. Measurement of commercial yoghurt samples

Sample Preparation

1mL supernatant through 0.22 µm filter → ultrafiltration → 100-fold dilution

It is shown in Figure 1 that the organic acids are well retained, and all have eluted within 22 minutes.

In Table 2, the calculated LOD is shown to be 2–4 orders of magnitude more sensitive than UV detection methods. Increased selectivity with SIM measurement made it possible to achieve faster and more accurate peak assignments.

The calibration curves shown in Figure 2 were used to analyze two actual samples in Figure 3. The two commercial yoghurts showed distinct differences in abundance of various organic acids, revealing differences in fermentation conditions, additives, and resulting flavors.

Conclusions

A method for quantitation of organic acids was developed with the combination of an Agilent Hi-Plex H column and a single quad LC/MS system. Testing confirms sensitivity and linear calibration. This analysis overcomes previous difficulties in achieving good retention and selectivity of organic acids without compromising sensitivity.

Real world samples of commercial yoghurt were successfully analyzed, demonstrating applicability for food, beverage, and similar fermented samples.

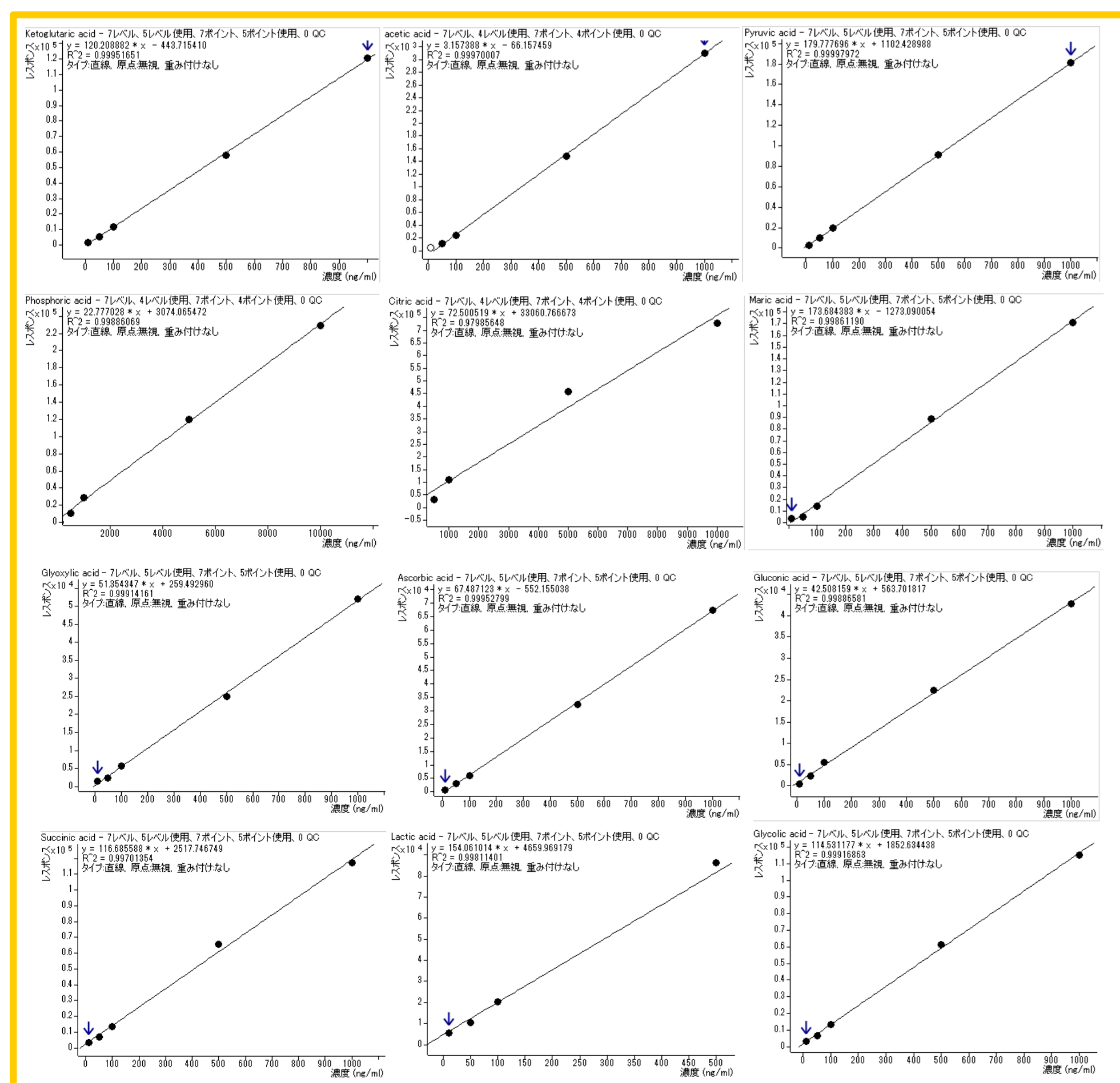


Figure 2. Calibration Curve