



Development of the Labor-Saving, Mercury-Free Analytical Method for the Quantification of Hop-Derived Polyfunctional Thiols in Beer

Toru KISHIMOTO ¹, Kazuhiko UEMURA ¹, Masayuki AIZAWA ¹, Kikuo SASAMOTO ², Nobuo OCHIAI ² (¹Asahi Breweries, Ltd., Ibaraki, Japan ²GERSTEL K.K., Tokyo, Japan)

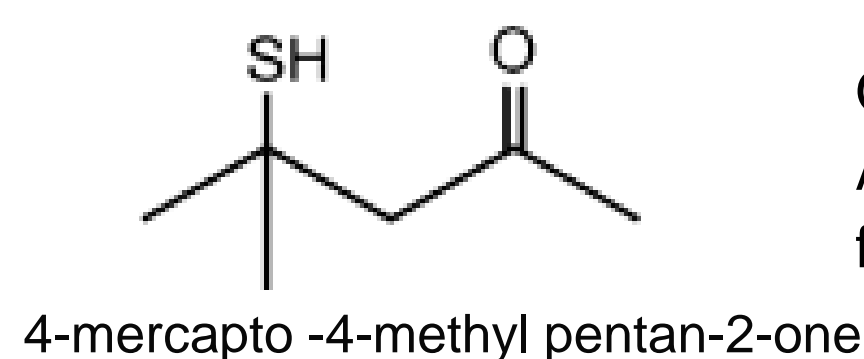
【Background】

Hop-derived polyfunctional thiols, e.g. 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH), and 3-mercaptohexyl acetate (3MHA), give high sensory impact on beer with their extremely low odor threshold value⁽²⁾.

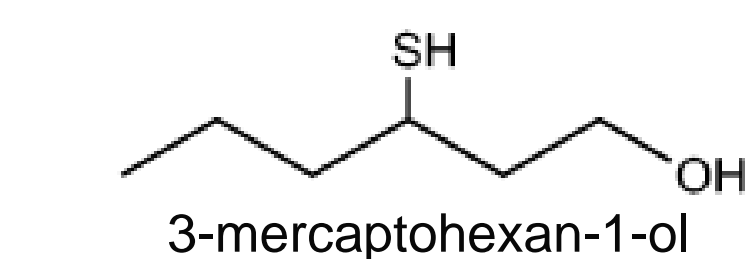
Besides the brewer's interests on these thiols, the conventional analytical method required heavy labor, hence the number of samples applied for the extraction in a day was limited.

Moreover, the existing method uses a reagent containing mercury ion (*p*-hydroxymercury benzoate : *p*HMB) ^(2,3,4), accompanied by the troublesome in the disposal of the waste solution. The growing of the world concern about environmental problems makes the disposal of mercury ion-containing wastes increasingly difficult.

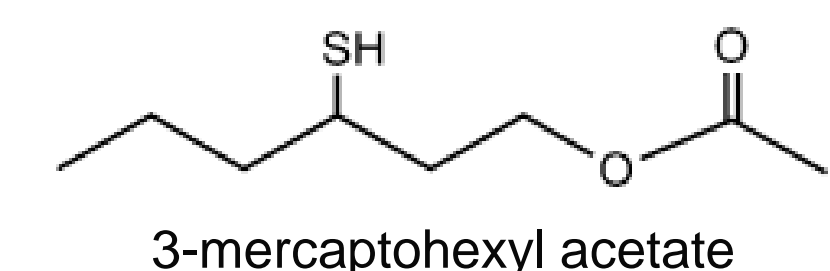
In this research work, we developed the labor-saving, mercury-free analytical method for the quantification of hop-derived polyfunctional thiols in beer.



Odor threshold in beer : 1.5 ng/L
Aroma description: blackcurrant, fruity, and catty.



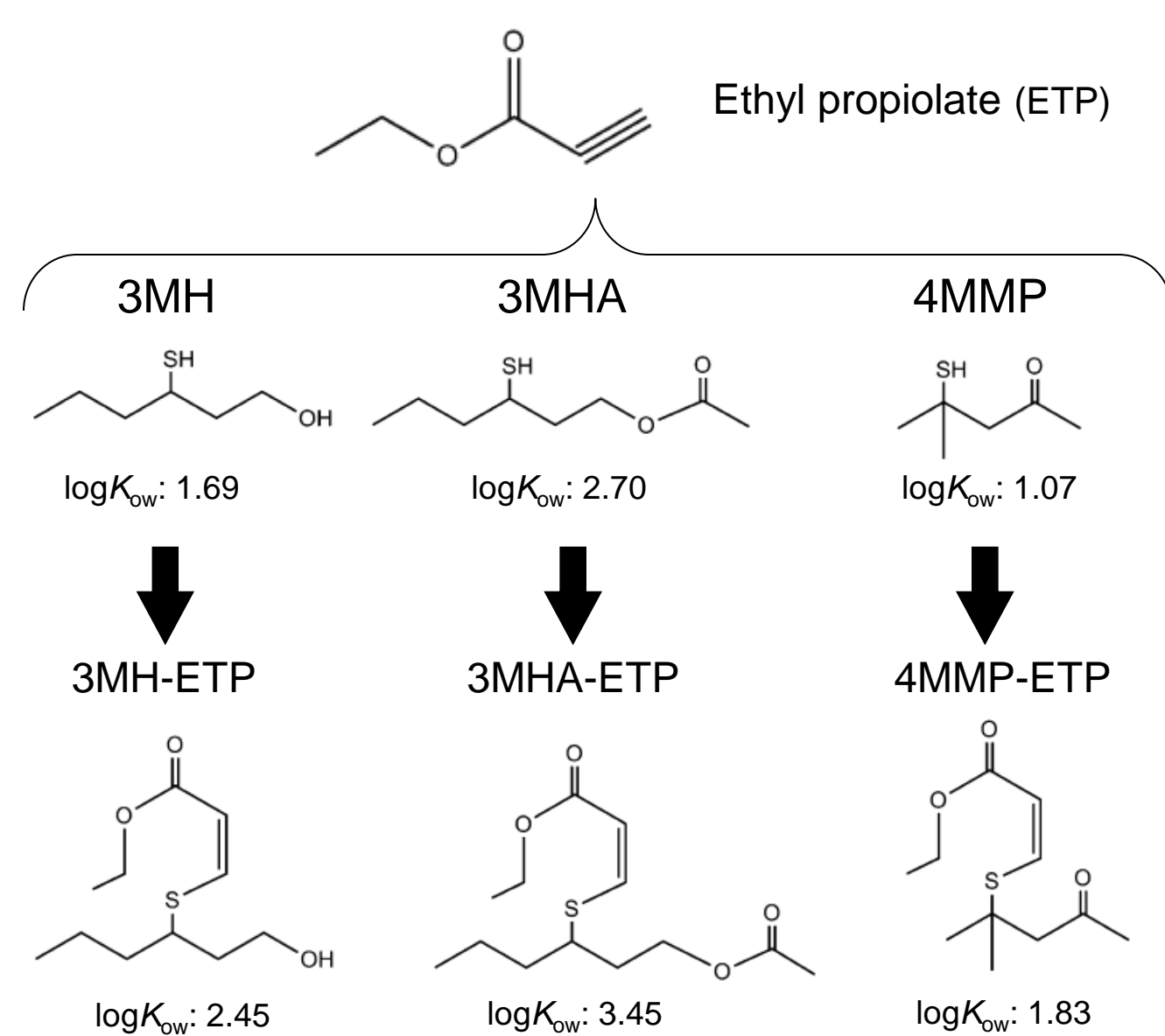
Odor threshold in beer: 55.0 ng/L
Aroma description: passion fruit, grape fruit, and catty.



Odor threshold in beer: 5.0 ng/L
Aroma description: passion fruit, fruity, and sweaty.

【Derivatization with ethyl-propiolate (ETP)】

Recently, Herbst-Johnstone et al reported the method for the quantification of varietal thiols in white wine⁽⁵⁾, that use ethyl propiolate (ETP) as a derivatising agent, followed by the extraction with SPE cartridge and the gas chromatography-mass spectrometry (GC-MS) analysis. The highly complicated matrices of beer hinder the ultratrace analysis of the compounds below threshold value. To achieve the ultratrace analysis of thiols in beer, we employ the extraction using stir bar sorptive extraction (SBSE) method, in-situ derivatization with ethyl propiolate, followed by the analysis using the triple quadrupole GC/MS (QQQ) system with thermal desorption unit.



【Newly Developed Method (1)】

Sample preparation

20 mL beer in 30 mL vial with internal standard d₁₀-4MMP and d₂-3MH

↓
alkalify the sample pH

↓
Addition of ethyl-propiolate (ETP)

↓
Addition of NaCl

↓
Extraction with

Stir Bar Sorptive Extraction (SBSE) method by stirring PDMS Twister (GERSTEL)

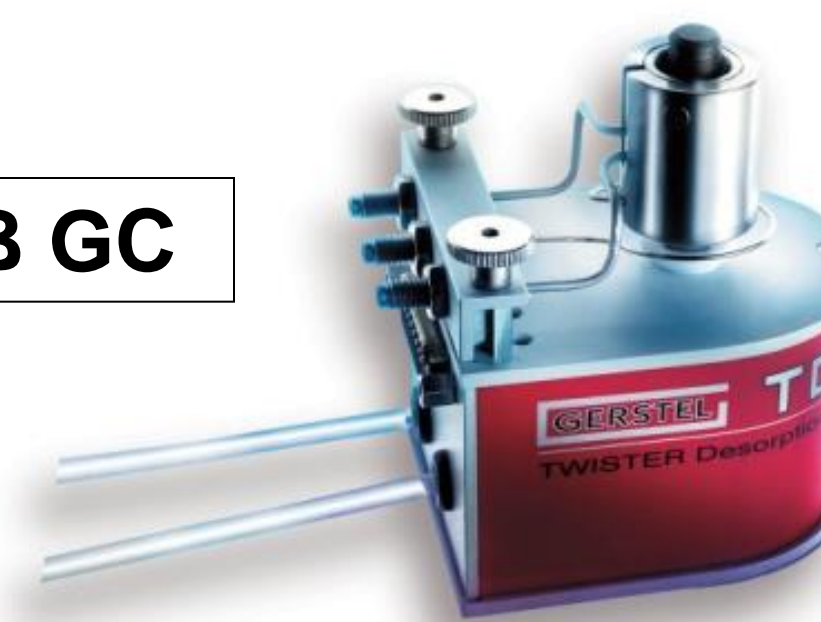


Sample Introduction

Thermal Desorption Unit (TDU :GERSTEL) with CIS4 inlet (GERSTEL)

Separation by Agilent 7890B GC

Detection



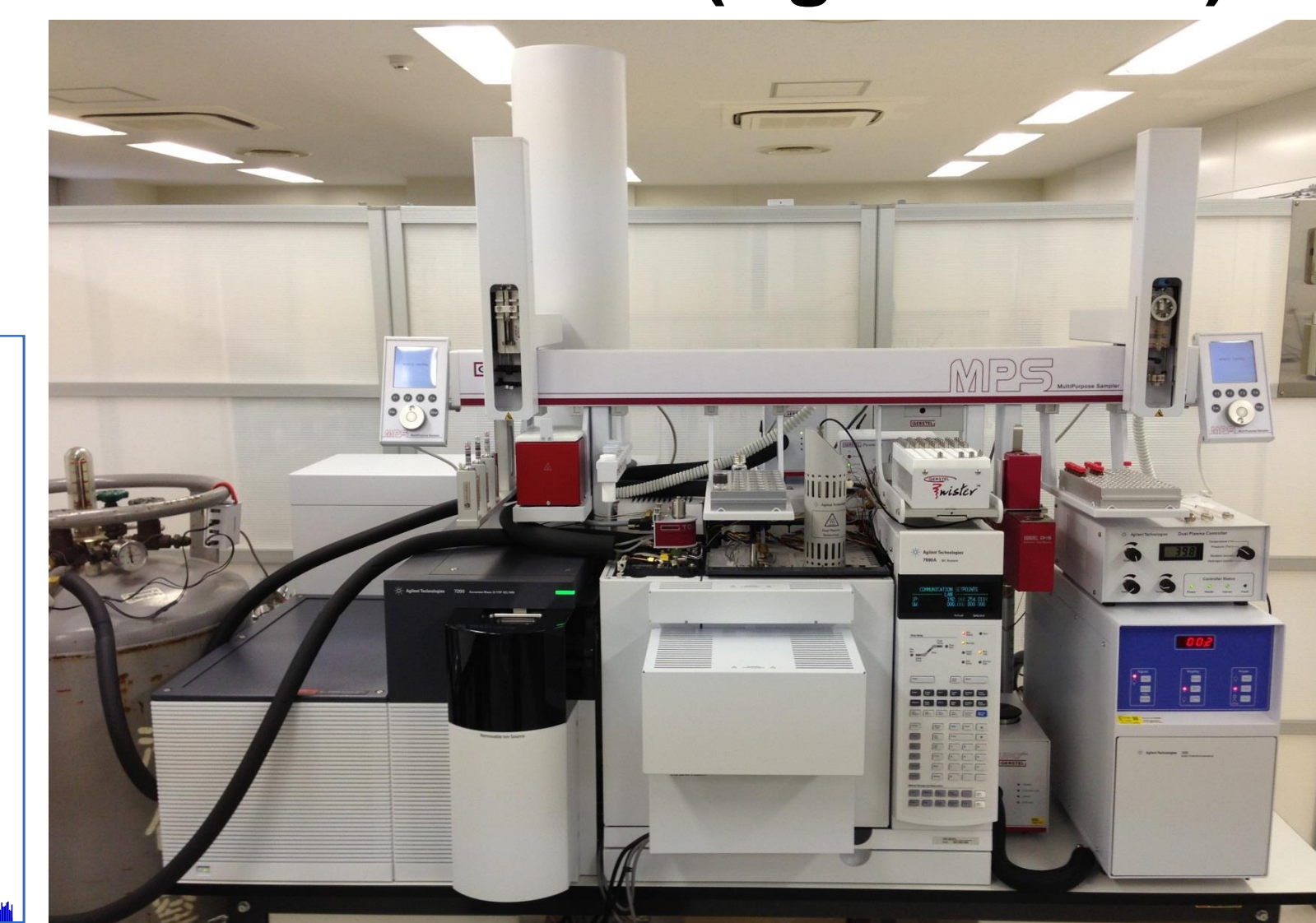
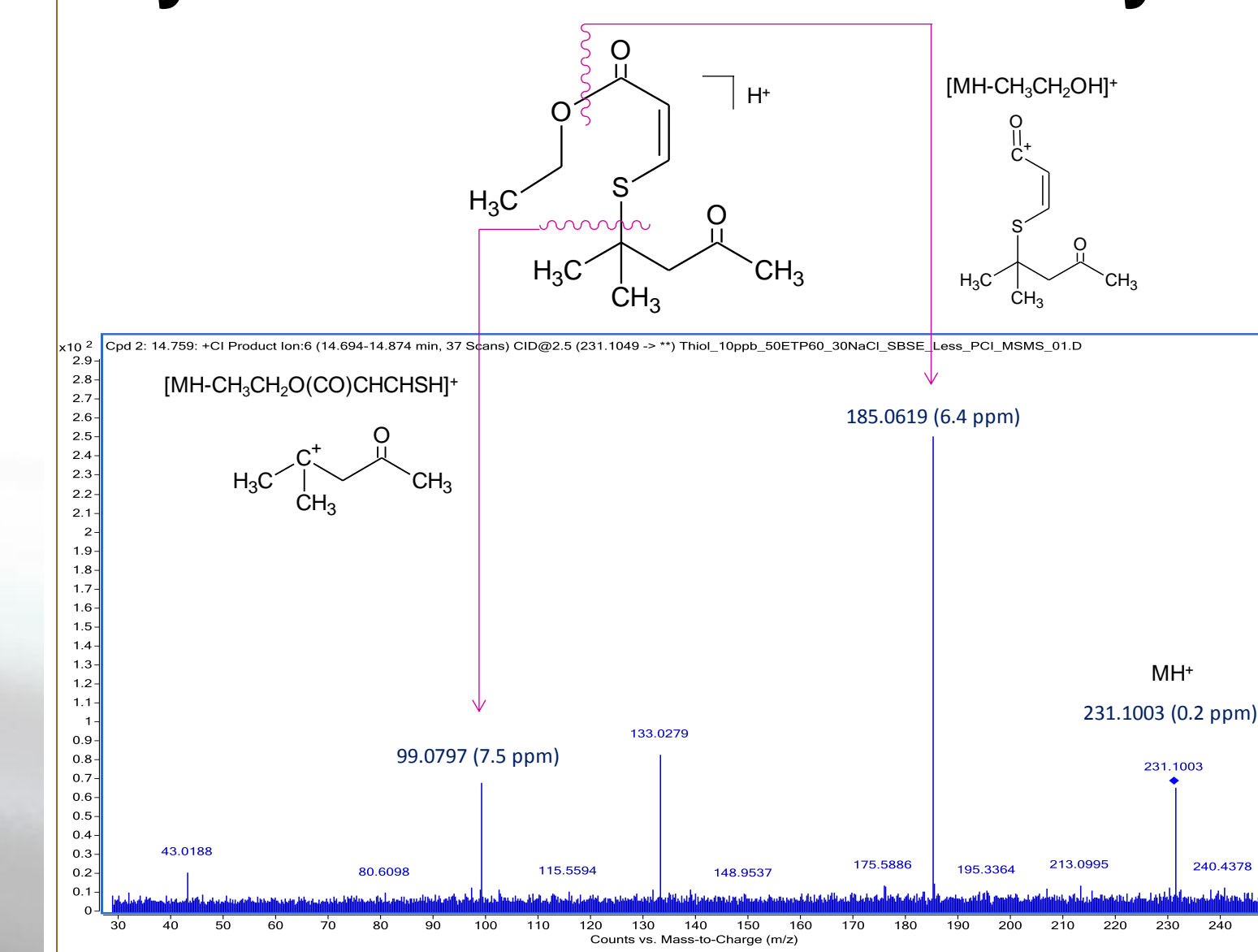
•Identification

Accurate mass analysis Q-TOF GC/MS (Agilent 7200) with product ion scan acquisition mode

•Quantification

Triple Quadrupole GC/MS System (GC/MS/MS: Agilent 7000B) with the SRM acquisition mode

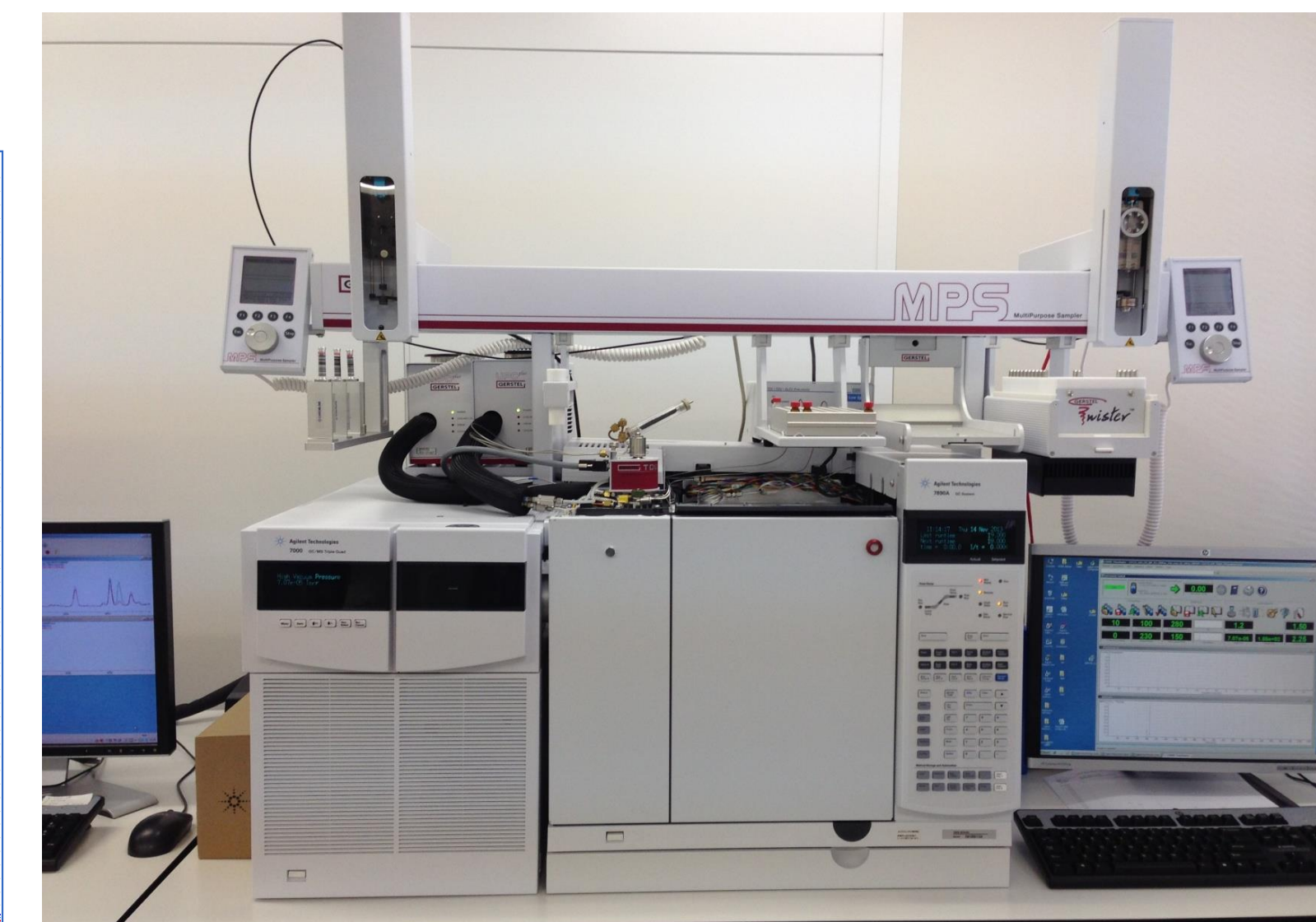
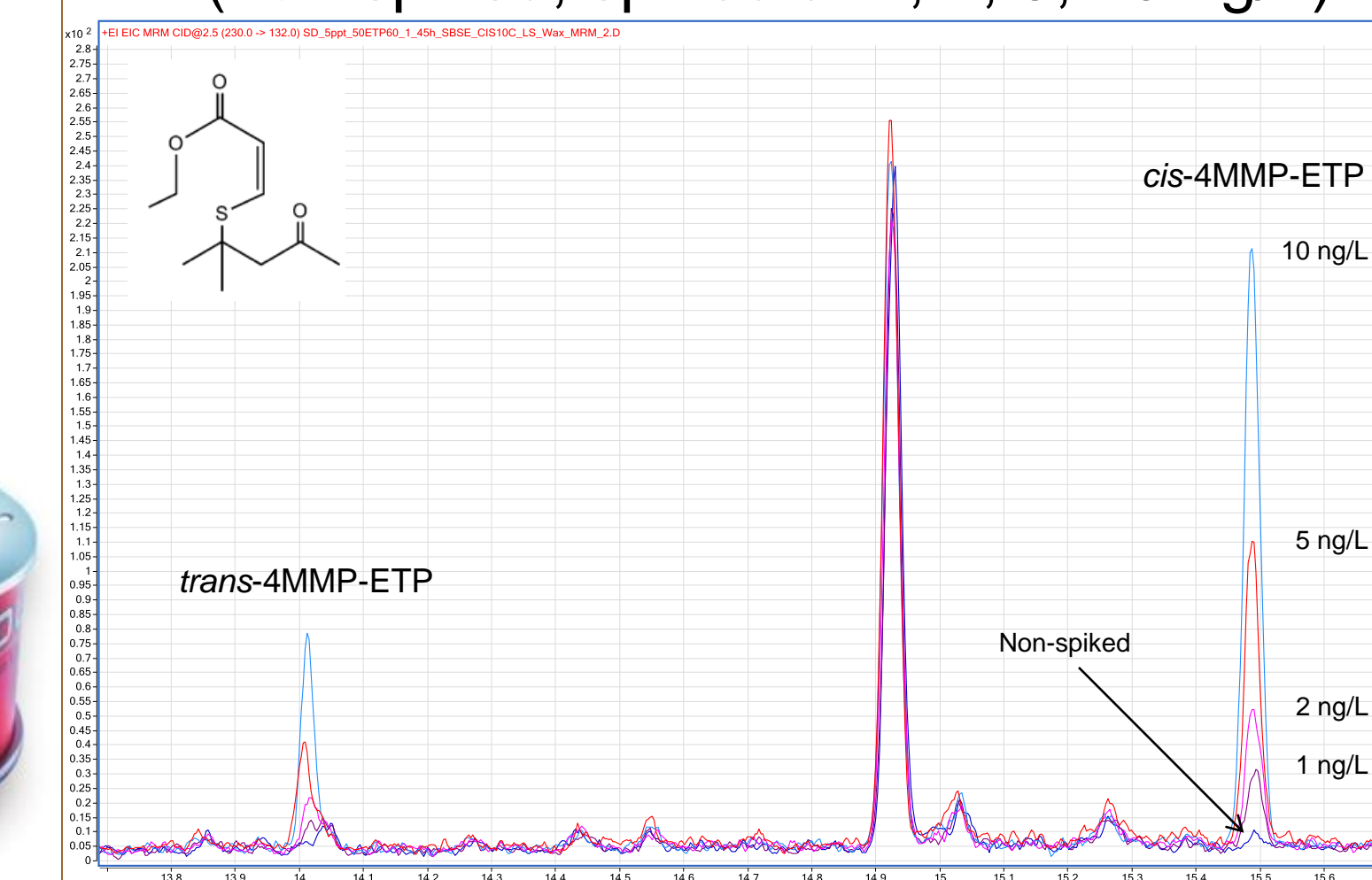
【 Identification and of ETP-Derivatized Polyfunctional Thiols by the Accurate Mass Analysis of Q-TOF GC/MS (Agilent 7200)】



TD-1D/2D GC-SCD/Q-TOF-MS (Agilent 7200)

【Quantification of ETP-Derivatized Polyfunctional Thiols by GC/MS/MS】

4MMP-ETP-derivative in beer (non-spiked, spiked at 1, 2, 5, 10 ng/L)



GC/MS/MS system: Agilent 7000B

Table 1. Linearity, LOD of *cis*-ETP derivatives of 4MMP, 3MH and 3MHA in beer. ⁽¹⁾

Compound	Quadrupole GC/MS (QQQ)			r ²	LOD (ng L ⁻¹)	RSD (%)
	Precursor ion	Product ion (target)	Product ion (Qual)			
cis-4MMP-ETP	230	132	99	0.9999	0.20	2.8
cis-3MH-ETP	232	152	141	0.9976	27	1.3
cis-3MHA-ETP	274	214	241	0.9943	0.19	7.2

【References】

- (1) Ochiai, N., Kikuo, S., Kishimoto, T., Analysis of hop-derived polyfunctional thiols in beer by stir bar sorptive extraction with in-situ derivatization and thermal desorption - gas chromatography - tandem mass spectrometry. *J. Chromatogr. A* 2014, in press
- (2) Kishimoto, T., Kobayashi, M., Yako, N., Iida, A., and Wanikawa, A. Comparison of 4-mercapto-4-methylpentan-2-one content in hop cultivars from different growing regions. *J. Agric. Food Chem.* 2008, 56, 1051-1057.
- (3) Tominaga T, Murat ML, Dubourdiou D. Development of a Method for Analyzing the Volatile Thiols Involved in the Characteristic Aroma of Wines Made from Vitis vinifera L. Cv. Sauvignon Blanc. *J. Agric. Food Chem.* 1998, 46, 1044-1048.
- (4) Gros, J., Nizet, S., and Collin, S. Occurrence of Odorant Polyfunctional Thiols in the Super Alpha Tomahawk Hop Cultivar. Comparison with the Thiol-rich Nelson Sauvignon Bitter Variety. *J. Agric. Food Chem.*, 2011, 59, 8853-8865
- (5) Herbst-Johnstone, M., Piano, F., Duhamel, N., Barker, D., Fedrizzi, B. Ethyl propiolate derivatization for the analysis of varietal thiols in wine. *J. Chromatogr. A*, 2013, 1312, 104-110.