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Simultaneous determination of γ-hydroxybutyrate and its precursor substances γ-butyrolactone and 1,4-butanediol in beverages using UHPLC-MS/MS

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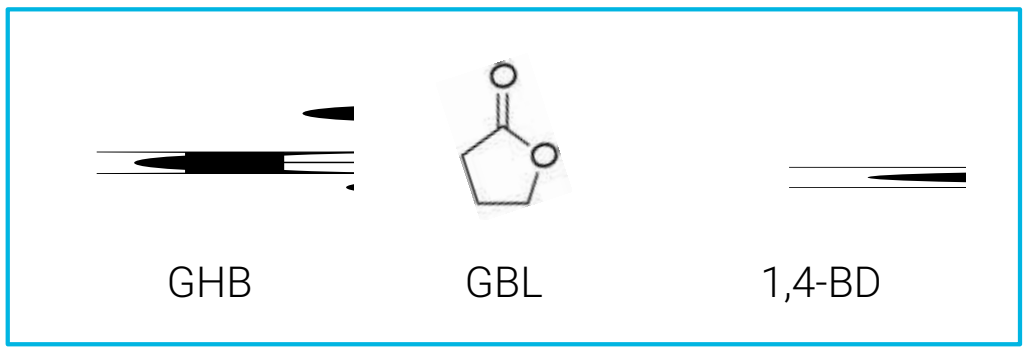
Introduction

γ-hydroxybutyrate (GHB) is a drug of abuse with a strong central nervous system depressant effect[1]. Recently, illicit use of GHB and its precursors, γ-butyrolactone (GBL) and 1,4-butanediol (1,4-BD), have become a serious social problem as related to drug-facilitated crimes, such as robberies, sexual assaults, and fraudulent gambling[2]. They are most commonly available on the street market or over the Internet and can be taken as a colourless, odourless liquid or white powder, tablet. GHB abuse has been reported in drug-facilitated sexual assaults (DFSA), because of its strong sedative and amnesic effects and can easily be added to drinks[3]. In this study, a rapid and accurate UHPLC-MS/MS method for the determination of GHB, GBL and 1,4-BD in beverages was developed. The established UHPLC-MS/MS method provides a robust tool for simultaneously determination of illegal addition of GHB and its precursor substances in beverages with excellent repeatability, good reliability, high sensitivity, which can be well used for the quality control in food industry.



Experimental

Sample Preparation

For dairy beverages and coffee drinks: accurately weigh 1.0 g sample to 10 mL centrifuge tube, add 4 mL of acetonitrile, mixed with a vertex shaker for 1 min, centrifuge at 6,000 rpm for 3 minutes, dilute 1 mL of supernatant with water to a final volume of 10 mL. Juice Drinks: measure 50 ml of sample and filter with filter paper. Then accurately weigh the filtrate (1.0 g) into a 10 mL volumetric flask and dilute to volume with water. For carbonated drinks, sonicate for 20 min to remove the gas in the beverage before weighing 1.0 g sample into a 10 mL volumetric flask. Then filtered the solution through a 0.22 µm filter and injected 2 µL into the UHPLC-MS/MS system.

Experimental

LC Conditions

Column	Agilent EclipsePlusC18, 3.0 mm × 150 mm, 1.8 µm ZORBA X LC column (p/n 959759-302)					
Flow rate	0.4 mL/min					
Column Temperature	40 ℃					
Injection	2 µL					
Mobile phase	A: H_2O with 0.02% Formic Acid; B: Methanol Gradient Elution					
	min	0	4.5	7.5	9.5	9.6
	B (%)	5	5	95	95	5

MS Conditions

The UHPLC system was connected to Agilent 6470 LC/TQ mass spectrometer for mass spectra acquisition. Samples were monitored via the ESI ionization mode and quantified by MRM model. MS conditions: drying gas 7 L/min at 300°C; nebulizer 35 psi; and sheath gas 11 L/min at 325°C.

MRM Transitions and Conditions

Compound	Prec. Ion	Prod. Ion	Frag. (V)	CE (V)	+/-
GHB	105.1	87.1	50	2	+
GHB	105.1	45.2	50	22	+
GHB	103.0	85.0	50	10	-
GHB	103.0	57.2	50	15	-
GBL	87.1	45.2	69	14	+
GBL	87.1	43.2	69	10	+
1,4-DB	91.1	73.1	40	2	+
1,4-DB	91.1	43.2	40	14	+
aminobutyric acid	104.1	87.1	59	10	+
aminobutyric acid	104.1	43.2	59	18	+

Method Optimization

Compound optimizations were performed by direct injection of GHB, GBL and 1,4-DB using ESI in both positive and negative modes. Parent and product ion transitions, Fragmentor and Collision Energy (CE) that were evaluated are summarized in Table.

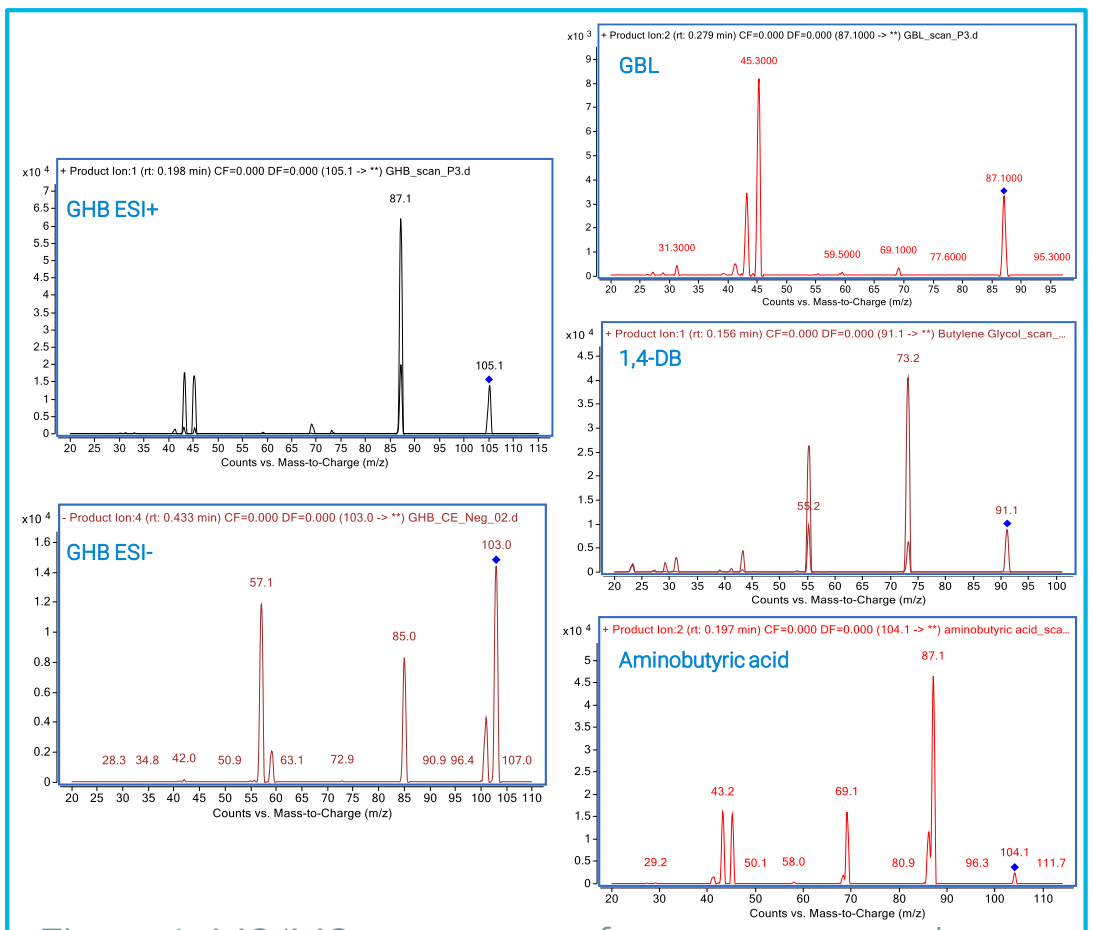


Figure 1. MS/MS spectrums of target compounds.

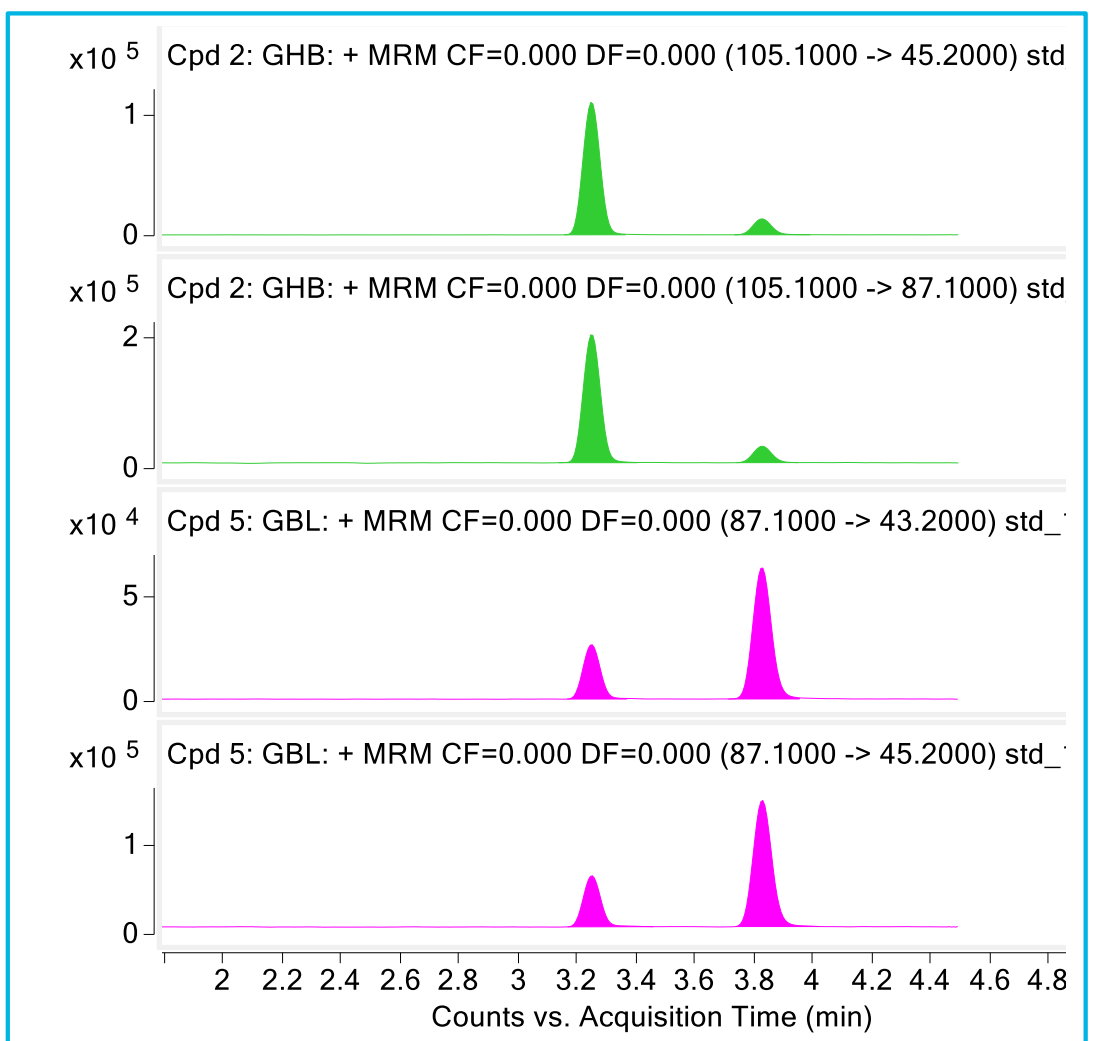


Figure 2. Chromatography of GHB and GBL.

In the positive ionization mode, protonated molecular ions were observed at m/z 105.1, m/z 87.1 and m/z 91.1 for GHB, GBL and 1,4-DB, respectively. The product ion spectrums of target compounds are shown in Fig. 1. For GHB, deprotonated molecules $[M-H]^-$ can be chosen as an alternative precursor ion in negative mode .

Moreover, it should be noted that under some conditions in ESI, the GHB molecule might lose water within the instrument source with formation of GBL. Therefore, in the ion chromatogram of GHB, a noticeable interference peak around 3.8 min was observed and it corresponded to the retention time of GBL, as show in Fig 2. And $[GBL+H_2O+H]^+$ also can be observed. It is of interest that the method can distinguish between in-source generated GBL or $[GHB-H_2O+H]^+$ and actual GBL in a sample. In this case, chromatographic separation of these two compounds was necessary in order to avoid co-elution. Peak resolution for GHB and GBL calculated from Fig. 2 was 5.4, which indicated a complete separation of the peaks.

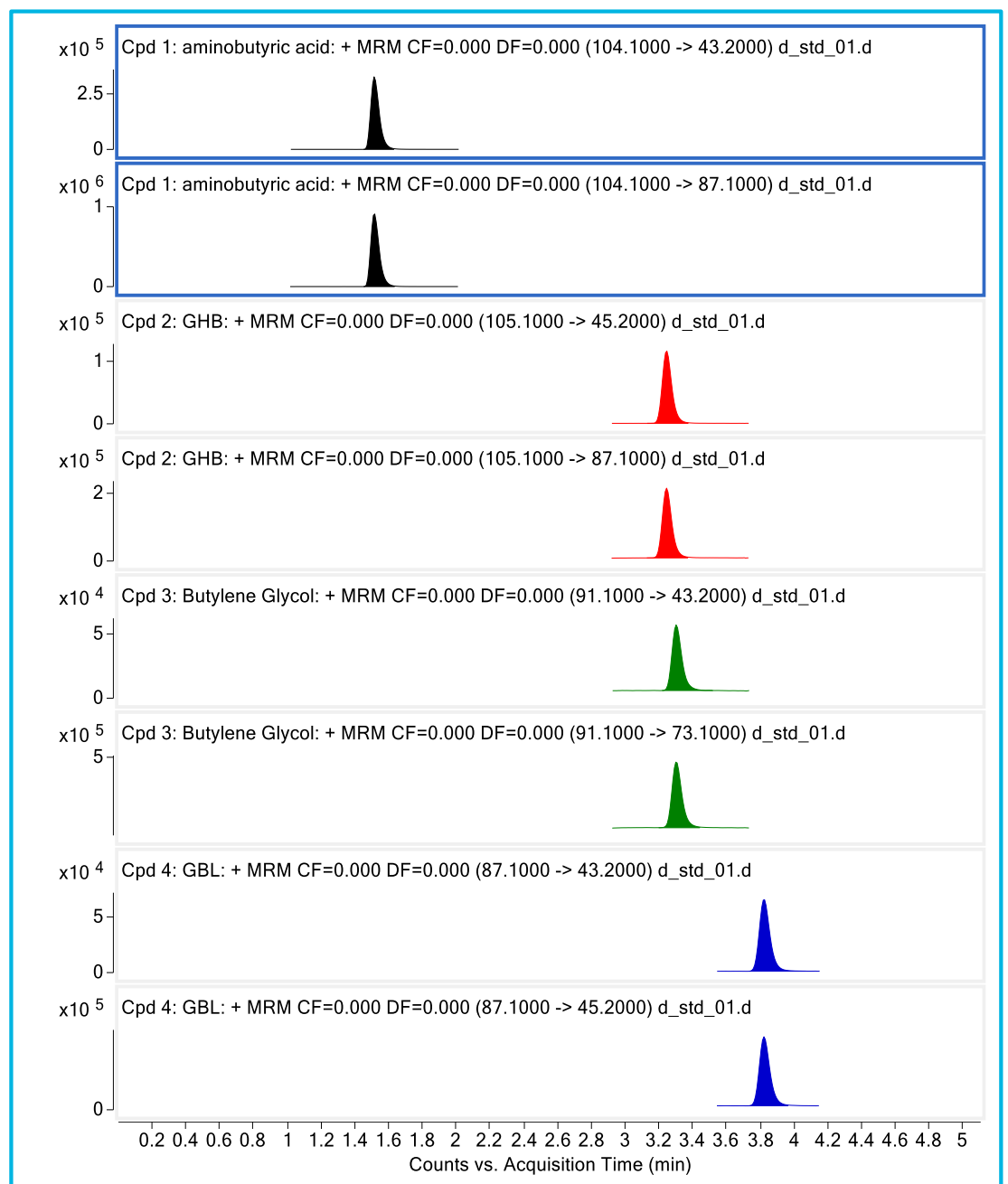


Figure 3. Separation of GHB, GBL, 1,4-DB and aminobutyric acid under optimized UHPLC-MS/MS conditions.

Results and Discussion

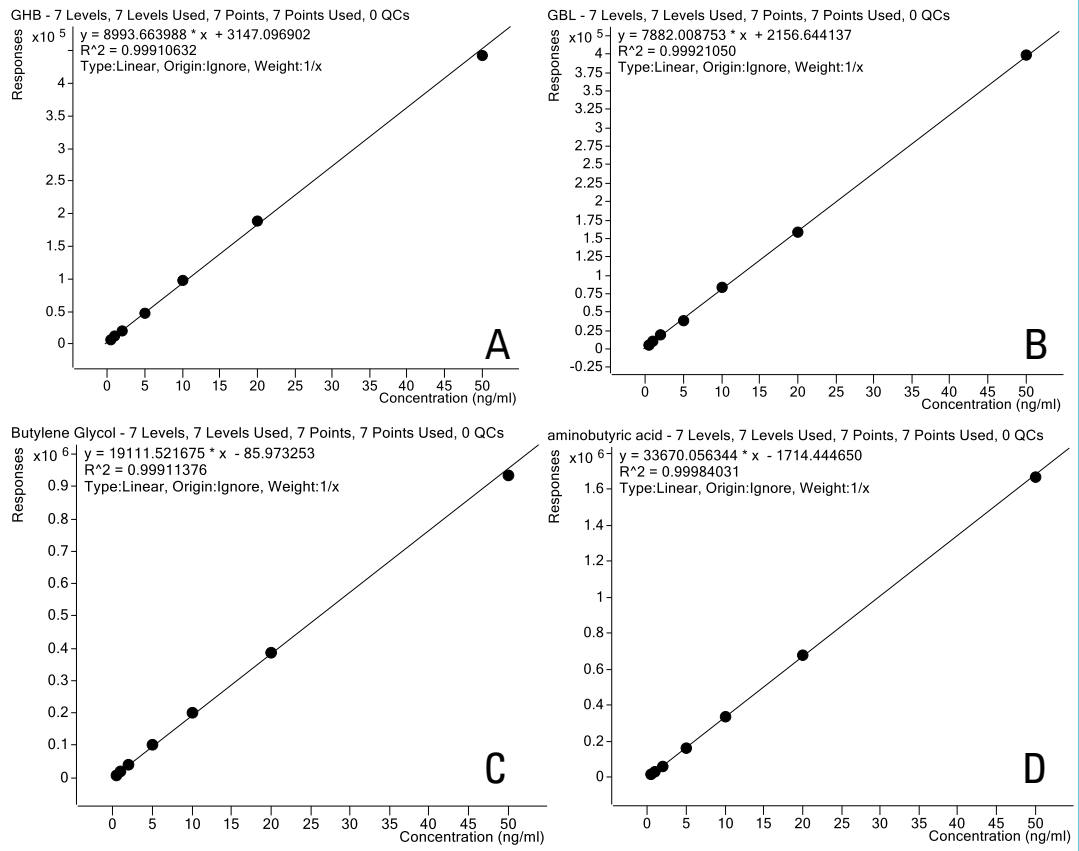


Figure 4. Calibration curves for GHB(A), GBL(B), 1,4-DB(C) and Aminobutyric acid(D) using solvent standards, linear fits $R^2 \geq 0.999$.

Conclusions

The LC/MS/MS method described here provides procedures for identification of multiple drugs of abuse in beverages with very fast analysis times. Sensitivity levels required are met and multiple reaction monitoring of several fragmentation transitions are carried out not only for quantitation using designated quantifying ions, but also for confirmation using designated qualifier ions. Using the Agilent C18 column with 1.8 µm particles allows for excellent resolution and peak shape at a relatively high flow rate of 400 µL/min for a 2.1 mm i.d. column and an ESI interface.

References

1. Soyoung Kang, Seung Min Oh, Kyu Hyuck Chung, et al. Journal of Pharmaceutical and Biomedical Analysis 98 (2014) 193–200.
2. Elisabetta Bertol, Francesco Mari, Fabio Vaiano, et al. Drug Testing and Analysis, 2015, 7(5): 376-384.
3. Elisabetta Bertol, Antonina Argo, Paolo Procaccianti, et al. Journal of Pharmaceutical and Biomedical Analysis 70 (2012) 518-522.

Method Performance Characterization

At the optimized sample preparation and chromatographic conditions, the separation of three target compounds was rapidly achieved within ten minutes, as show in Fig 3. Based on the optimization of Fragmentor Voltage, Collision Energy and Nozzle Voltage, two MRM transitions are used for the quantifier and qualifier for each compound with $[M+H]^+$ as precursor ions, respectively. The established quantitative method demonstrates excellent accuracy (recoveries at 3 spiked levels in eight kinds of beverages are between 88.9~108.2% for three target compounds), excellent repeatability (RSDs are between 1.08 - 9.83%, n=5), good linearity ($R^2 > 0.99$), and excellent sensitivity that easily meets regulatory needs in food industry.

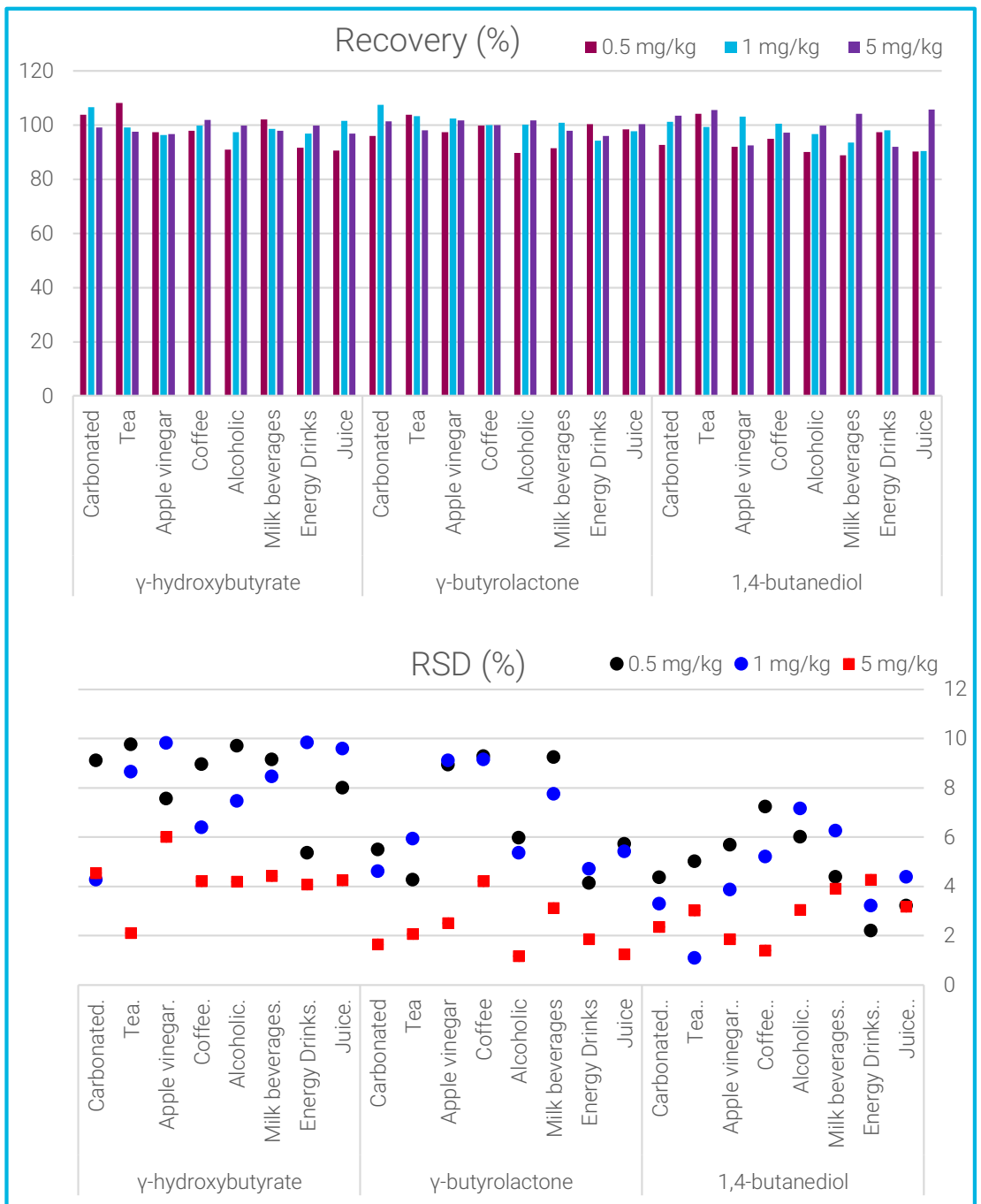


Figure 5. Recovery(%) and RSD(%) of GHB, GBL and 1,4-DB in different beverages.

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