SHIMADZU
Excellence in Science

Application News

Gas Chromatograph Brevis™ GC-2050

Dual-Column Analysis of Blood Alcohol Content (BAC) with Brevis GC-2050

H. Miyabayashi, E. Kobayashi, E. Shimbo

User Benefit

- Employing two different types of columns makes BAC analysis more productive.
- Employing Brevis GC-2050 achieves less occupied space in the laboratory.
- Employing Hydrogen is also available in this system instead of Helium. This makes the total run-time quite shorter.

■ Introduction

Analysis of blood alcohol content (BAC), as well as other volatile organic compounds (VOCs) analysis, is essential technique in forensic field to determine the degree of intoxication caused by alcohol consumption and to evaluate criminality.

Generally, this analysis is performed with gas chromatograph (GC) coupled with headspace sampler (HS) and frame ionization detector (FID). To get accurate results, this analysis needs two columns for cross-check. Both accuracy and quickness are required in this analysis, so connecting a single HS-GC system to two columns leading to each FID fulfills the requests with remarkably smart way.

Our new GC named Brevis GC-2050 is designed to be more compact than ever, which can be utilized for this BAC analysis as well as Nexis™ GC-2030 that is our high-end model. The width of GC-2050 is approximately 35% smaller than existing GC model. Moreover, GC-2050 can hold two columns despite its smaller size, which contributes more productivity in the laboratory as well. This application news introduces BAC analysis with HS-20 NX and Brevis GC-2050 (Fig.1). Using the specialized column series SH-BAC PLUS, linearity and repeatability of ethanol were evaluated, as well as separation of VOCs. Hydrogen is employed as carrier gas in this system, which achieves faster runtime.

* Sterile sheep blood which is commercially available was used in this experiment.

Fig. 1 Brevis™ GC-2050 and HS-20 NX
(Specially designed space-saving transfer line for GC-2050)

Table 1 Consumables list for column equipment

ID in Fig. 2,3	Name	P/N	Note
①	2-hole Ferrule	225-19056	10 pc per unit
②	Twin MS Kit	225-20201-91	10 pc per unit
③	SMI Union Ferrule	227-35025-02	0.4 mm - 0.5 mm, 10 pc per unit
④	Restrictor Tubing	227-35023-02	Cut a 2 m tube into two sets of 100 mm

■ Preparation of the Instrument

In this experiment, two different types of columns were equipped to HS, which are connected to two FIDs, respectively (shown in Fig. 2). Simultaneous dual-lines analysis achieves higher throughput as well as qualification accuracy. SH-BAC PLUS1 (30 m × 0.32 mm I.D., 1.8 µm) and SH-BAC PLUS2 (30 m × 0.32 mm I.D., 0.6 µm) were used for analytical columns.

Table 1 shows the list of consumables for this dual-column analysis, and Fig. 3 shows the schematic illustration image of the system. Both columns are connected to "Restrictor Tubing" (0.22 mm I.D.、100 mm) with "SMI Union Ferrule" (0.4 mm - 0.5 mm). "2-hole Ferrule" and "Twin MS Kit" were utilized in nut connection part in HS. 0.5 mL loop (P/N: 225-21889-85) was set for sample loop. Hydrogen and Nitrogen were employed as carrier gas and as pressuring gas, respectively.

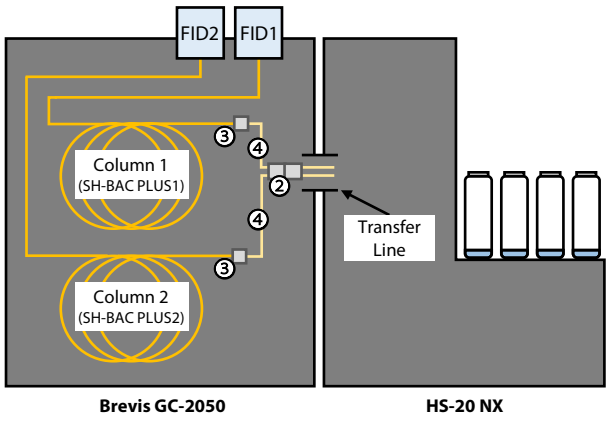

Fig. 2 Schematic illustration of HS-GC dual-column system

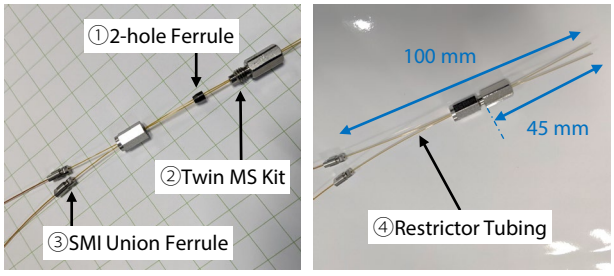

Fig. 3 Column connection modules for the system

■ Analytical Condition

Table 2 shows the analytical condition.

Table 2 Analytical condition

GC Model	: Brevis GC-2050
Headspace Sampler	: HS-20 NX
[HS-20 NX]	
Oven Temp.	: 65 °C (20min)
Sample Line Temp.	: 90 °C
Transfer Line Temp.	: 100 °C
Vial Stirring	: 3
Vial Pressurization Time	: 0.5 min
Pressure Equalization Time	: 0.1 min
Loading Time	: 0.5 min
Load Equalization Time	: 0.1 min
Injection Time	: 0.5 min
Needle Flush Time	: 3 min
GC cycle Time	: 4 min
Vial Pressure	: 80 kPa, Nitrogen
Sampling Volume	: 0.5 mL
[GC-2050]	
Carrier Gas	: Hydrogen
Carrier Gas Control	: Constant Linear Velocity (60 cm/sec)
Purge Flow	: 0 mL/min
Injection Mode	: Split 1:13
Column	: SH-BAC PLUS1 (P/N：227-36260-01) (30 m × 0.32 mm I.D., 1.8 µm) SH-BAC PLUS2 (P/N：227-36263-01) (30 m × 0.32 mm I.D., 0.6 µm)
Column Temp.	: 40 °C (3 min)
FID Temp.	: 250 °C
FID H2 Flow Rate	: 32 mL/min
FID Make up Flow Rate	: 24 mL/min, Nitrogen
FID Air Flow Rate	: 200 mL/min

■ Sample Preparation

Fig. 4 shows the schematic illustration for how to put the sample to the HS vial. 480 µL of water or blood was induced in 20 mL HS vial, followed by adding 20 µL of spiking standard solution and 100 µL of internal standard (IS) solution. The lid was hermitically sealed. For practical experiment, adding 500 µL of blood and 100 µL of IS solution are assumed. Table 3 shows the concentration of each standard solution.

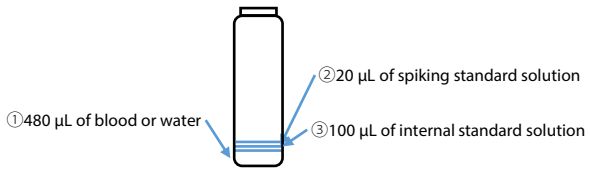

Fig 4 Scheme of sample preparation

Table 3 Concentration of each standard solution

Compound	Stock solution conc.	Final conc. (except IS solution)
Spiking standard solution for calibration curve (Ethanol)	600 mg /mL	20 mg /mL
	3000 mg /mL	100 mg /mL
	6000 mg /mL	200 mg /mL
	12000 mg /mL	400 mg /mL
IS solution（tert-Butanol）	600 mg /mL	100 mg /mL

■ Analysis Process

Fig. 5 shows schematic illustration of an example of analytical process. For better repeatability, a highly concentrated sample was injected for conditioning prior to the whole experiment. Highly polar compounds like Ethanol and Methanol are likely to adsorb to the sample line. It causes underestimation of the peak area for several initial injections and the peak area typically becomes higher during serial injections. Therefore, filling the adsorption points up with high concentrated compounds beforehand will promotes the analytical stability.

As a high concentrated sample, each 1000 mg/dL of Ethanol and tert-Butanol was used. After injecting a high concentrated sample, two serial injections of water were performed as blanks. It was confirmed that no carryover was detected during the blank analysis.

After this pre-conditioning process, five points calibration samples were analyzed, followed by a blood sample. For checking the stability of the peak area, 100 mg/dL of standard Ethanol sample was analyzed after every five blood injections.

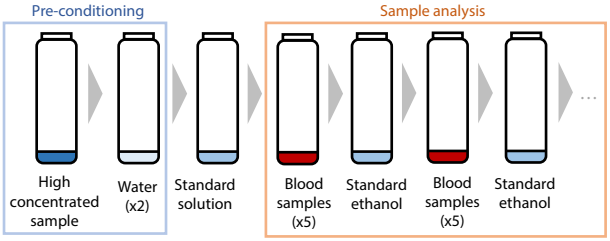

Fig. 5 Recommended analytical process

■ Linearity of the Calibration Curve

Fig. 6 shows the calibration curve that was created with mean values of six repeated analysis of each calibration points. Difference was evaluated when using water and blood as solvent. Both Ethanol and Butanol peaks were counted higher in blood solvent than water solvent, and the slope of the calibration was slightly different from each other. This should be because the matrix compounds containing in blood should interfere the liquid-gas equilibrium condition inside the vial. The effect should differ from compounds. Correlation coefficients for both water and blood calibration curve were more than 0.999.

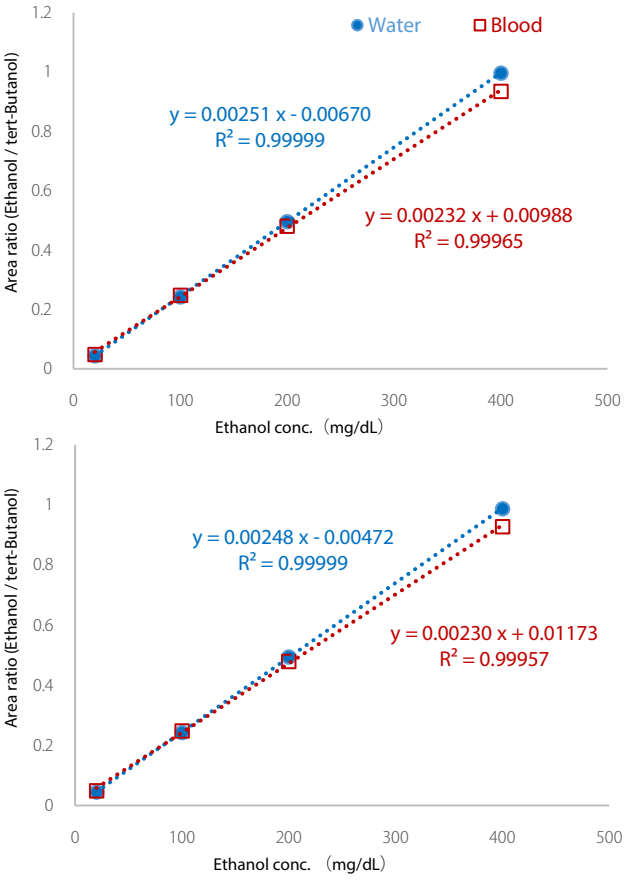

Fig. 6 Calibration curve of Ethanol
(Upper: SH-BAC PLUS1, Lower: PLUS2)

■ Analysis of Blood Sample

Blood was assumed as the field sample and was added to the standard solutions shown in Table 3 (Final conc. of 0~400 mg/dL except IS solution). The obtained chromatograms are shown in Fig. 7. The quantification results of these samples are shown in Table 4. Errors from the spiked concentration were all within 10%, even under using either calibration curve whose solvent is water or blood. Although blood calibration curve should be preferable, water calibration curve should be also available under the difference was fully evaluated like shown in this experiment.

Repeatability of the 100 mg/dL standard solution analyzed after every five blood samples analysis was also evaluated. The %RSD was 1.29 (n=6) that is satisfying result.

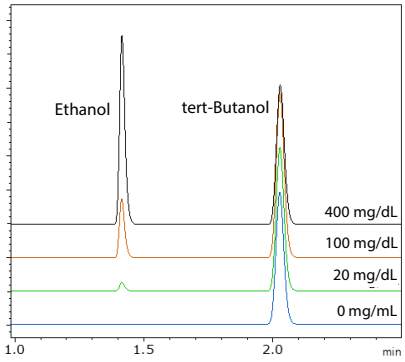

Fig. 7 Chromatograms of blood samples that contains both spiking and internal standards

Table 4 Difference of the obtained concentration values from the spiked concentration values (n=5)

Final conc. inside the vial (except IS solution)		Water calibration curve		Blood calibration curve	
		Conc. (mg/dL)	Error (%)	Conc. (mg/dL)	Error (%)
20 mg/dL	SH-BAC PLUS1	19.2	-4.0	18.5	-7.5
	SH-BAC PLUS2	18.7	-6.5	18.3	-8.5
100 mg/dL	PLUS1	98.9	-1.1	105.0	+5.0
	PLUS2	98.2	-1.8	104.7	+4.7
200 mg/dL	PLUS1	192.2	-3.9	206.4	-3.2
	PLUS2	190.7	-4.7	205.2	+2.6
400 mg/dL	PLUS1	373.6	-6.6	403.6	+0.9
	PLUS2	370.0	-7.5	400.1	+0.0

■ VOCs Separation

Seven VOCs (Methanol, Ethanol, 1-Propanol, 2-Propanol, Acetone, tert-Butanol and Acetaldehyde) that are typically identified in blood sample were analyzed. Resulted chromatograms from each 20 mg/dL (Final concentration in the vial) is shown in Fig. 8. All the compounds were eluted within 2.4 min and were fully separated. Accurate qualification should work because different separation pattern was obtained with each column. Table 4 shows repeatability (n=20) of the retention time and of the peak area ratio to tert-Butanol (internal standard) of each compound.

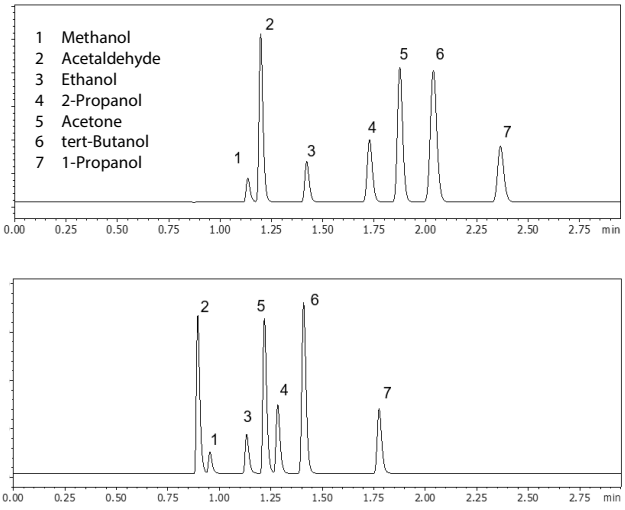

Fig. 8 Chromatogram of seven VOCs
(Upper：SH-BAC PLUS1, Lower：SH-BAC PLUS2)

Table 5 %RSD of the retention time (RT) and peak area ratio (n-=20)

ID	Compound	SH-BAC PLUS1		SH-BAC PLUS2	
		RT	Peak area ratio	RT	Peak area ratio
1	Methanol	0.0066	0.63	0.0165	0.57
2	Acetaldehyde	0.0077	1.24	0.0082	0.95
3	Ethanol	0.0067	0.48	0.0121	0.46
4	2-Propanol	0.0069	0.38	0.0080	0.47
5	Acetone	0.0060	0.70	0.0101	0.36
6	tert-Butanol	0.0062	-	0.0104	-
7	1-Propanol	0.0066	0.38	0.0010	0.36

■ Summary

BAC analysis was performed with Brevis GC-2050 coupled with HS-20 NX. Pre-conditioning kept the system stable and helped to obtain satisfying repeatability. The blood sample also worked to obtain adequate repeatability and calibration linearity.

This experiment employed Hydrogen as carrier gas instead of Helium. Rapid, compact, and reliable analytical system was accomplished by this analysis system.

01-00570-EN First Edition: Sep. 2023

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/

SHIMADZU
Excellence in Science

Gas Chromatography

Application News

Blood alcohol content (BAC) analysis using a liquid injection approach

R. Kelting[1], M. Tabak[2]
1 Shimadzu Europa GmbH, 2 Shimadzu Benelux B. V.

User Benefits

- Blood alcohol analysis using liquid injection is an alternative to the headspace analysis of blood samples for clinical purposes
- It provides reliable results for whole blood and plasma samples without adverse matrix effects

■ Introduction

Blood alcohol content (BAC) is relevant for legal regulations regarding driving limits, but also investigated for forensic and clinical purposes with respect to poisoning. Blood alcohol analysis does not only focus on ethanol, which is the classical drinking alcohol, but also investigates other alcoholic substances like methanol and isopropanol as well as metabolites like acetone. Ethanol is present in alcoholic beverages, but also used for antiseptics, various daily life products, and pharmaceuticals. Being a central nervous system depressant, consumption can cause severe intoxication with toxic levels at above 100 mg/dL blood [1]. Methanol intoxication could originate from the consumption of counterfeit alcohol, with as few as 30 mL pure methanol consumed being potentially lethal [2]. Isopropanol is widely used as solvent or antiseptic in households and sometimes used as cheap substitute for ethanol by alcohol abusers. It is metabolized by alcohol dehydrogenase to acetone, which alike isopropanol is a central nervous depressant [3].

Blood alcohol analysis can be performed using gas chromatography (GC) with flame-ionization detection (FID). Due to the complexity of blood as matrix, headspace analysis is a widely used technique for simple sample transfer onto the GC column, which is especially valuable in forensics, where the matrix states investigated can drastically vary depending on the time the sample is taken. For clinical analysis, fresh blood is used, making the sample more uniform. In this approach, the analysis of BAC using a liquid injection technique as alternative to headspace sampling is presented.

■ Sample Preparation and Calibration

The blood samples are analyzed twice for blood alcohol content: Methanol and ethanol content are determined from the whole blood, whereas isopropanol and acetone are analyzed in the blood plasma. In both cases dilution in serum is used to ensure the blood samples are within the calibration range. This spans concentrations of 100 to 2000 mg/L for methanol, isopropanol, and acetone, and concentrations of 200 to 4100 mg/L for ethanol. n-propanol is used as internal standard and water as matrix. Despite analyzing part of the targets from whole blood and part from plasma, one analytical method and the same data processing for all compounds are used, facilitating data evaluation and routine work.

■ Results

Fig. 1 shows exemplary calibration curves for the four target compounds methanol, ethanol, isopropanol, and acetone proving excellent linearity.

Compound quantification is not affected by the whole blood and plasma matrices, all peaks are reliably detected at the lowest level in both matrices (Fig. 2).

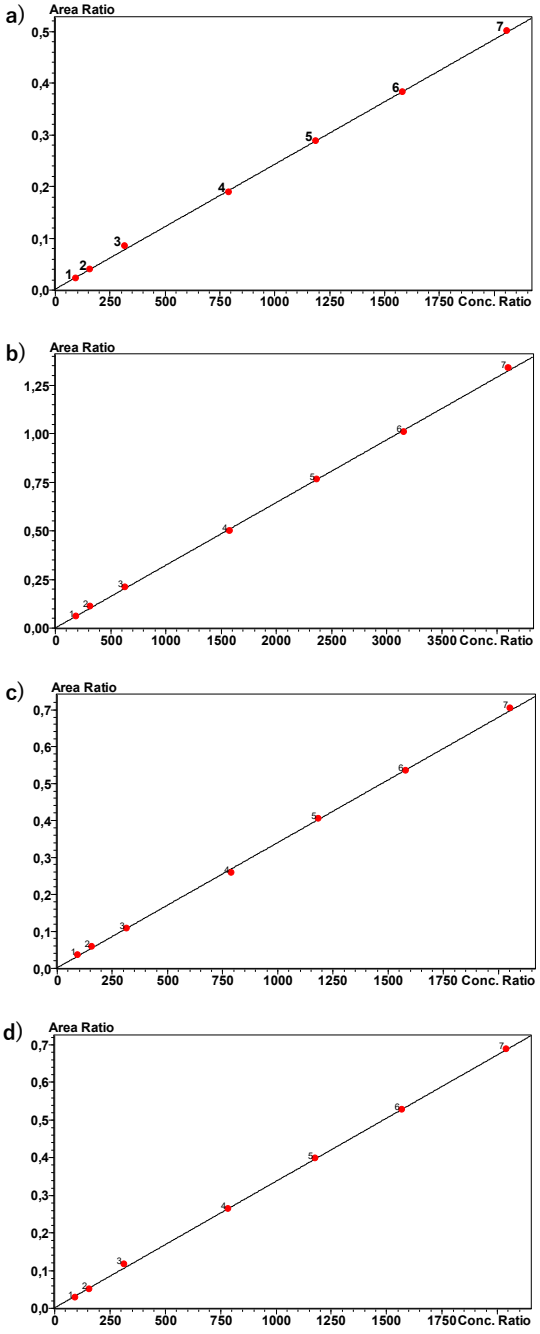

Fig. 1 Exemplary calibration curves for a) methanol, b) ethanol, c) isopropanol, and d) acetone

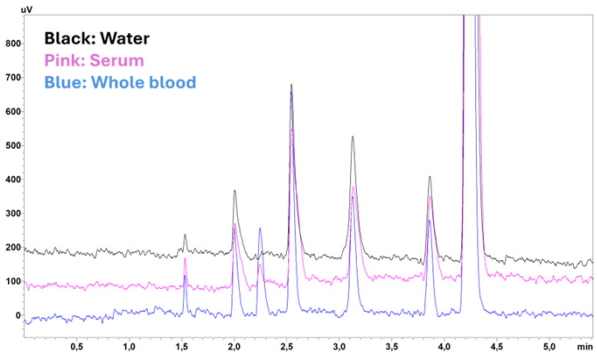

Fig. 2 Compound signals at the lowest level in water (black), serum (pink), and whole blood (blue)

Reproducibility of retention time and area for each compound are summarized in table 1 for compound concentrations of 300 mg/L ethanol / 150 mg/L other compounds and 3200 mg/L ethanol / 1600 mg/L other compounds, determined from 5 consecutive measurement series. The values for ethanol and methanol refer to whole blood as matrix, in which these two compounds are analyzed, whereas the values for isopropanol and acetone refer to plasma as matrix, as quantification of these compounds is performed via blood plasma samples.

Table 1 %RSD retention time and %RSD area (n = 5)

Name	Level 1*		Level 2*	
	% RSD Ret. Time	% RSD Area	% RSD Ret. Time	% RSD Area
Methanol	0.1	4.0	0.2	2.2
Ethanol	0.1	1.9	0.1	2.4
Isopropanol	0.2	3.1	0.2	2.3
Acetone	0.1	3.7	0.1	3.3

* Level1: 400 mg/L Ethanol, 200 mg/L others; Level 2: 4000 mg/L Ethanol, 2000 mg/L others

■ The Package

The recommended analytical hardware and software configuration is listed below.

❑ *Main Unit*

Nexis GC-2030 with FID-2030: Gas chromatograph with flame ionization detector

❑ *Accessory*

AOC-30i autosampler: Liquid autosampler with 30 vials capacity

❑ *Main Consumables*

SH-BAC1 column (30 m x 0.32 mm x 1.8 µm;

P/N 221-76135-30)

❑ *Software*

LabSolutions LCGC

Fig. 3 Nexis GC-2030 equipped with FID-2030 detector and AOC-30i autosampler

■ Conclusion

Liquid sample injection presents a reliable alternative to headspace injection when analyzing blood samples for clinical purposes. Ethanol and methanol can be determined from whole blood, isopropanol and acetone from plasma samples without adverse matrix effects. Both whole blood and plasma analysis can be done using the same analytical method and one data processing approach, facilitating data evaluation and routine work.

■ Acknowledgements

The methodology reported in this application was developed and evaluated by the Klinisch Farmaceutisch Laboratorium (KFL) of ETZ TweeSteden Hospital in Tilburg (Netherlands).

■ References

[1] D. Perry, D. Klimaszyk, Z. Kolacinski, J, Szajewski, "Ethyl Alcohol (Ethanol)" in *McMaster Textbook of Internal Medicine*. Available: https://empendium.com/mcmtextbook/chapter/B31.II.20.2.1

[2] D. Perry, D. Klimaszyk, Z. Kolacinski, J, Szajewski, "Methyl Alcohol (Methanol)" in *McMaster Textbook of Internal Medicine*. Available: https://empendium.com/mcmtextbook/chapter/B31.II.20.2.2

[3] D. Perry, D. Klimaszyk, Z. Kolacinski, J, Szajewski, "Isopropyl Alcohol" in *McMaster Textbook of Internal Medicine*. Available: https://empendium.com/mcmtextbook/chapter/B31.II.20.2.4.

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/

SHIMADZU Europa GmbH,
www.shimadzu.eu

05-SCA-180-051-EN First Edition: June. 2024