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Validation Report: Available Carbohydrates Assay Kit (cat. no. K-AVCHO)

1. Scope

Megazyme's Available Carbohydrates Assay Kit (K-AVCHO) is an enzymatic method suitable for the measurement of available carbohydrates (AVCHO) comprising total digestible starch (TDS) plus maltodextrins, sucrose, D-glucose, D-fructose and lactose. This method was developed in-house and measures each sugar in g/L for liquids or as g/100g for solid samples. The sum of each sugar gives the available carbohydrate content. This method is currently awaiting official method recognition.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by Available Carbohydrates Assay Kit (K-AVCHO).

3. Performance characteristics

The selectivity, working range, limit of detection, limit of quantification, trueness (*bias*) and precision of this kit is detailed in this report.

3.1. Selectivity

The assays specifically measure D-glucose, D-fructose and D-galactose derived from digestible starch plus maltodextrins, sucrose and lactose, as well as free D-glucose and D-fructose.

Interfering substances in the sample being analysed can be identified by including an internal standard. Quantitative recovery of this standard would be expected. Losses in sample handling and extraction are identified by performing recovery experiments, i.e. by adding D-glucose, D-fructose and, or, D-galactose to the sample in the initial extraction steps.

3.2. Working Range

Assay follows the Available Carbohydrates Assay Kit (K-AVCHO) standard procedure. 0.1mL of D-glucose, D-fructose plus D-galactose mixed standard was used as sample, with a range of concentrations (0.04-0.8 g/L total sugars) which corresponds to 4-80 µg of total sugars per cuvette.

Absorbance A2 was taken 3 min after the addition of the 1st trigger enzyme (GalDH/GalM), giving the measurement of D-galactose.

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Absorbance A3 was taken 5 min after the addition of the trigger enzyme (HK/G6P-DH), giving the measurement of D-glucose.

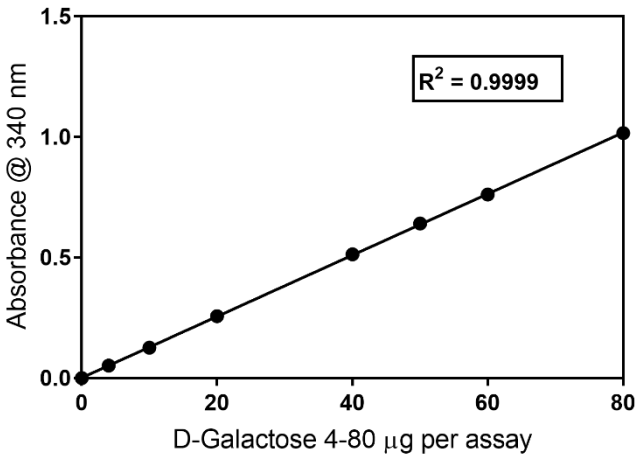
Absorbance A4 was taken 10 min after the addition of the trigger enzyme (PGI), giving the measurement of D-fructose.

Available Carbohydrates = to the sum of D-Galactose + D-glucose + D-fructose.

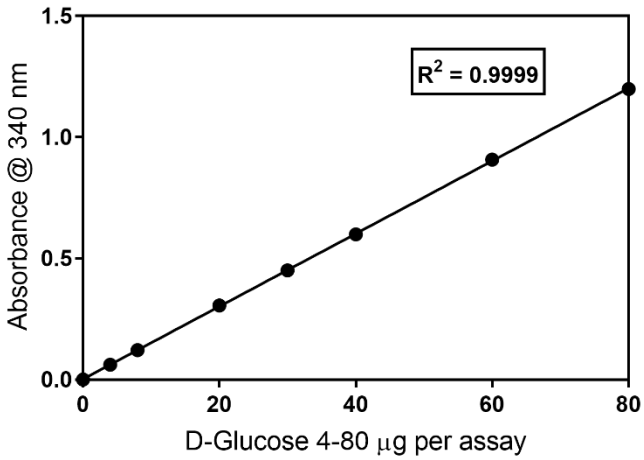
All absorbances were read at 340nm and at 30°C as recommended in the procedure.

The working range is linear between 4-80 µg of total sugars per assay.

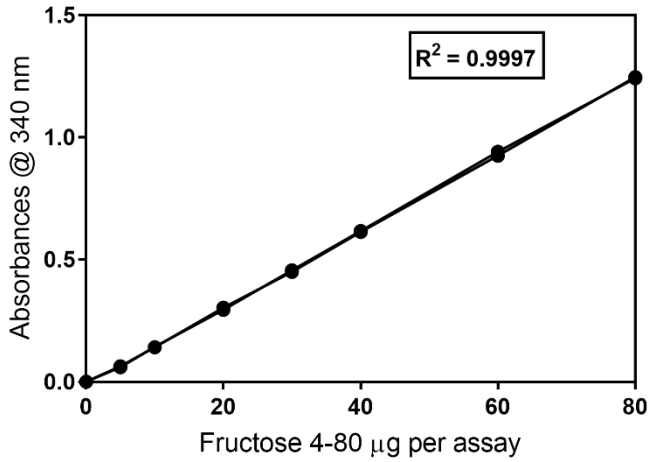
D-Galactose Standard Curve



D-Glucose Standard Curve



D-Fructose Standard Curve



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3.3. LOD and LOQ

The **instrument limit of detection** for the supplied control powder (bottle 9), using method 1-0.5 g of sample (measured as glucose) is 1.475 g/100 g, which is derived from an absorbance difference of 0.02 with an extract volume of 20.5 mL.

The **calculated limit of detection (LOD)** and the calculated **limit of quantification (LOQ)** for this report purpose is based on the analysis of samples that have been taken through the whole Available Carbohydrates Assay Kit (K-AVCHO) procedure.

- The LOD is the lowest concentration of the analyte that can be detected by the method. LOD is calculated as 3 x s'0; where s'0 is the standard deviation of a number of samples A1 reading.
- The LOQ is the lowest level at which the kit's performance is acceptably repeatable. LOQ is calculated as kQ x s'0; where s'0 is the standard deviation of a number of samples A1 reading. The IUPAC default value for kQ is 10

- For Available Carbohydrates Assay Kit (K-AVCHO)

  LOD – For 1.0 mL of sample (maximum volume)
  D-Galactose = 0.069 mg/L
  D-Glucose = 0.070 mg/L
  D-Fructose = 0.141 mg/L

  LOQ – For 1.0 mL of sample (maximum volume)
  D-Galactose = 0.138 mg/L
  D-Glucose = 0.209 mg/L
  D-Fructose = 0.352 mg/L

  LOD – For 0.5 g sample weight and extract volume of 20.5 mL (measured as glucose)
  Available Carbohydrates Control = 0.07377 g/100g

  LOQ – For 0.5 g sample weight and extract volume of 20.5 mL (measured as glucose)
  Available Carbohydrates Control = 0.29509 g/100g

* **Note:** The above detection limits are for samples as used in the assay, after sample preparations if required (e.g. deproteinisation). The dilution used in pre-treatment must be accounted for while establishing the detection limits for specific samples.

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3.4. Trueness (*Bias*)

Comparison of the mean of the results (x) achieved with Available Carbohydrates Assay Kit (K-AVCHO) method with a suitable reference value (x ref). For this report, Relative Bias is calculated in per cent as:

***b(%)* = x – xref / xref x 100.**

The reference material for this purpose is D-galactose, D-glucose and D-fructose, supplied with the Available Carbohydrates Assay Kit (K-AVCHO) at 0.2 g/L of each sugar. Relative Bias has also been calculated for the kit control supplied with the kit - the value of the control used for the purpose of this report is 45.5 g/100 g of available carbohydrates measured on an "as is" basis.

Relative Bias *b(%)*

	n	Ref Material (g/L)	Mean (g/L)	*b*(%)
D-Galactose	9	0.2	0.2044	2.222
D-Glucose	18	0.2	0.2049	2.472
D-Fructose	17	0.2	0.2008	0.383

	n	Ref Material (g/100g)	Mean (g/100g)	*b*(%)
Avail. carbohydrates	18	45.5	44.98	-1.132

3.5. Precision

This report details the reproducibility of the Available Carbohydrates Assay Kit (K-AVCHO), it is a measure of the variability in results, on different days and by different analysts, over an extended period of time.

For the purpose of this report different lot numbers of the kit standard are used as the reference material, for D-galactose, D-glucose and D-fructose, and one lot number of the kit control at 45.5g/100g of available carbohydrates is used as the reference material for available carbohydrate content.

Reproducibility

	n	Ref Material (g/L)	Mean (g/L)	Standard Deviation	%CV
D-Galactose	9	0.2	0.2044	0.0048	2.35
D-Glucose	18	0.2	0.2049	0.0026	1.26
D-Fructose	17	0.2	0.2008	1.1950	1.39

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	n	Ref Material (g/100g)	Mean (g/100g)	Standard Deviation	%CV
Avail. carbohydrates	18	45.5	44.98	1.1950	2.66

Repeatability of this kit has been assessed using a range of sample types. Assay followed the procedure as outlined in the Available Carbohydrates Assay Kit (K-AVCHO).

This is a measure of the variability in results where:

a all results are presented as available carbohydrates on a dry weight basis;

b on each day samples of each material were analyzed in duplicate;

c RSDr of the available carbohydrates assay was assessed using 8 samples. The available carbohydrate content of the samples tested covered a working range of 44.1 to 88.9% (w/w). The repeatability (%RSD_r) across this sample data set was less than or equal to 3.58% for all samples.

Sample	Available Carbohydrates, % (w/w)[a], mean[b] ±2 SD, (%RSD_r[c] %)				Interday mean, ±2 SD, (%RSD_r)
	Day 1	Day 2	Day 3	Day 4	
Wheat Starch	87.3 ± 2.1	90.2 ± 2.2	88.2 ± 0.6	90 ± 1.1	88.9 ±2.8
	1.21	1.21	0.34	0.61	1.60
All Bran	43.2 ± 2.2	45.4 ± 0.4	43.2 ± 1	44.5 ± 0.2	44.1 ±2.2
	2.55	0.40	1.20	0.27	2.53
Sweet Potato	59.6 ± 0.2	60.7 ± 1.3	58.2 ± 2.1	60.4 ± 1	59.7 ±2.3
	0.14	1.10	1.80	0.81	1.92
Ripe Banana	65.4 ± 0.4	70 ± 0.3	67.1 ± 1	66.8 ± 0.6	67.3 ±3.6
	0.28	0.19	0.77	0.46	2.68
Carrot	53.7 ± 0.9	57.4 ± 0.6	55.1 ± 1.5	55.3 ± 0.3	55.4 ±2.9
	0.87	0.54	1.36	0.23	2.58
Red Pepper	51 ± 0.5	55.4 ± 2.5	53.8 ± 3.1	52.9 ± 1.9	53.2 ±3.8
	0.49	2.25	2.92	1.83	3.58
Ryvita	60.6 ± 1.3	61.5 ± 1.3	61 ± 2.4	62.6 ± 0.7	61.4 ±2
	1.07	1.04	1.96	0.60	1.61
Swede	55 ± 4.3	53.6 ± 0.4	54.2 ± 1.3	54.1 ± 2	54.2 ±2.1
	3.92	0.34	1.19	1.82	1.96

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Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of D-galactose, D-glucose and D-fructose as Available Carbohydrates in various matrices. Data presented in this report verifies and validates that this method is fit for the purpose intended, which is summarised below

Validation Summary	D-Galactose	D-Glucose	D-Fructose	Avail Carbohydrate Control.
Working range (µg in cuvette)	4-80	4-80	4-80	4-80
LOD (mg/L)	1.4	1.4	0.2	0.074 g/100g
LOQ (mg/L)	4.2	5.6	0.5	0.295 g/100g
Relative Bias *b*(%)	0.894	-0.284	-0.425	-1.132
Reproducibility (%CV)	0.76	0.87	0.24	2.66