



**White Paper:** Comparative study of glucose/fructose testing in honey with an assay kit applied to the semi-automated analyser RX misano requiring minimal sample preparation

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## **INTRODUCTION**

Recent developments in the honey industry have resulted in an increased need for reliable analytical methods to monitor the quality specification of honey and more specifically, to measure the levels of glucose and total sugars present. In response to this, Randox Food Diagnostics have collaborated with Geomiel to conduct a study using the RX misano to determine glucose and total sugars levels present in a blind sample assessment.

Honey quality control criteria are specified in many regulations including the Codex Alimentarius Standard 12-1981 and European Directive 2001/1/10/EC which is now being implemented worldwide.

An assay kit has been developed for the determination of D-glucose and D-fructose in honey samples. The assay kit can be used manually but it can be also applied to the semi-automated analyser RX misano, which allows automatic processing and reporting. By using this system, honey samples can be analysed within 6 minutes (D-glucose) and 16 minutes (D-glucose + D- fructose) with assay ranges 3g/100g to 75g/100g respectively. This comparative study reports the data obtained after blind sample assessment by two laboratories using this system, samples were also assessed by two external confirmatory laboratories using other methodologies.

## **METHODOLOGY**

The Glucose/Fructose kit was used for the determination of D-glucose, D-fructose and D-glucose + D-fructose (System RX misano, Kit: GF2635, Randox Food Diagnostics, Crumlin, UK). The experimental procedure was performed according to manufacturer's instructions for use. The method is based on enzymatic reactions and the concentration of sugars is directly proportional to the increase in absorbance measured spectrophotometrically at 340nm. The assay kit was applied to the semi-automated analyser RX misano and included ready-to-use reagents and standard solution. Liquid honey samples (1g) were dissolved in deionised water (100mL) and 5µL was used for sample analysis. Samples were also externally determined by a UK based laboratory using chromatography with EC detector and a German based laboratory using mass spectrometry.

## **RESULTS**

Three blind honey samples were analysed by four different laboratories to determine sugar profile in each sample: glucose, total sugars (glucose + fructose) and fructose. Two out of the four laboratories applied the kit and system in question: Randox Food Diagnostics (RFD) HQ laboratory RX misano Serial No: 051-16-00023 Kit GF2635 Lot: 395217 and Geomiel Laboratory RX misano Serial No: 051-16-00036 Kit GF2635 Lot: 395217 (System RX misano Lab 1, System RX misano Lab 2). The other two laboratories are accredited confirmatory laboratories: UK based and German based (Confirmatory Lab 1, Confirmatory Lab 2 respectively).

The results showing the glucose profile, total sugars profile and fructose profile of each honey sample are displayed in sections 1, 2 and 3 respectively.

Each section shows the concentrations identified by each laboratory for the respective sugar (Tables 1, 5, 9), the accuracy for each sample displayed against Confirmatory Lab 1 and Lab 2 (Tables 2, 6, 10). Additional tables show System RX misano Lab 2 inter-assay precision (Tables 3, 7, 11) and overall inter-laboratory precision (Tables 4, 8, 12).

**Section 1 Glucose Profile**

Table 1. Reported Glucose Concentrations (g/100g)

	System RX misano Lab 1 (RFD HQ )	System RX misano Lab 2 (Customer Site)	Confirmatory Lab 1 (UK)	Confirmatory Lab 2 (Germany)
Honey Sample	Conc. g/100g	Conc. g/100g	Conc. g/100g	Conc. g/100g
1	26.8	25.4	26.3	27.7
2	33.8	32.5	33.1	34.4
3	30.9	29.9	29.8	33.3

Table 2. Accuracy of glucose

	System RX misano Lab 1 (RFD HQ )	System RX misano Lab 2 (Customer Site)	Confirmatory Lab 1 (UK)
Honey Sample	Recovery (%)	Recovery (%)	Recovery (%)
1	97	92	95
2	98	94	96
3	93	90	89
	System RX misano Lab 1 (RFD HQ )	System RX misano Lab 2 (Customer Site)	Confirmatory Lab 2 (Germany)
Honey Sample	Recovery (%)	Recovery (%)	Recovery (%)
1	102	97	105
2	102	98	104
3	104	100	112

Table 3. System RX misano Lab 2 (Customer Site) 'Inter-assay' precision

Honey Sample (n=2)	CV (%)
1	0.28
2	0.22
3	0.24

Table 4. Overall 'Inter-laboratory' precision

Honey Sample	CV (%)
1	3.62
2	2.47
3	5.25

**Section 2 Total Sugar Profile**

Table 5. Reported total sugars (glucose + fructose) concentrations (g/100g)

	System RX misano Lab1 (RFD HQ)	System RX misano Lab 2 (Customer Site)	Confirmatory Lab 1 (UK)	Confirmatory Lab 2 (Germany)
Honey Sample	Conc. g/100g	Conc. g/100g	Conc. g/100g	Conc. g/100g
1	66.3	62.2	67	68.1
2	74.1	67.6	73.4	74.6
3	70.4	66.1	69.4	74.3

Table 6. Accuracy of total sugars

	System RX misano Lab 1 (RFD HQ)	System RX misano Lab 2 (Customer Site)	Confirmatory Lab 1 (UK)
Honey Sample	Recovery (%)	Recovery (%)	Recovery (%)
1	97	91	98
2	99	91	98
3	95	89	93
	System RX misano Lab 1 (RFD HQ)	System RX misano Lab 2 (Customer Site)	Confirmatory Lab 2 (Germany)
Honey Sample	Recovery (%)	Recovery (%)	Recovery (%)
1	99	93	102
2	101	92	102
3	101	95	107

Table 7. System RX misano Lab 2 (Customer Site) 'Inter-assay' precision

Honey Sample (n=2)	CV (%)
1	0.23
2	1.26
3	0.11

Table 8. Overall 'Inter-laboratory' precision

Honey Sample	CV (%)
1	3.91
2	4.49
3	4.82

### Section 3 Fructose Profile

Table 9. Reported fructose concentrations (g/100g)

	System RX misano Lab 1 (RFD HQ)	System RX misano Lab 2 (Customer Site)	Confirmatory Lab 1 (UK)	Confirmatory Lab 2 (Germany)
Honey Sample	Conc. g/100g	Conc. g/100g	Conc. g/100g	Conc. g/100g
1	39.5	36.9	40.7	40.4
2	40.3	35.2	40.4	40.2
3	39.5	36.4	39.7	41.0

Table 10. Accuracy of fructose

	System RX misano Lab1 (RFD HQ)	System RX misano Lab 2 (Customer Site)	Confirmatory Lab 1 (UK)
Honey Sample	Recovery (%)	Recovery (%)	Recovery (%)
1	98	91	101
2	100	88	100
3	97	89	97
	System RX misano Lab1 (RFD HQ)	System RX misano Lab 2 (Customer Site)	Confirmatory Lab 2 (Germany)
Honey Sample	Recovery (%)	Recovery (%)	Recovery (%)
1	97	91	99
2	100	87	100
3	100	92	103

Table 11. System RX misano Lab 2 (Customer Site) 'Inter-assay' precision

Honey Sample (n=2)	CV (%)
1	0.19
2	2.62
3	0.39

Table 12. Overall 'Inter-laboratory' precision

Honey Sample	CV (%)
1	4.39
2	6.54
3	5.00

### CONCLUSION

Similar sugar concentrations generated for each honey sample were obtained by four different laboratories using different methodologies. Results show when applying the same lot of

glucose/fructose kits using two different systems and operators there is excellent recovery (%) (87-104%) comparable to confirmatory laboratories across all sugar profile, similarly when comparing each confirmatory laboratories recovery to each other recovery (%) falls within (89-112%). Customer's inter-assay precision for all three samples for each different sugar produced a CV (%) <3% which demonstrates the repeatability and performance of the assay in the hands of the end user. Likewise, inter-laboratory precision produced from four laboratories ran on three different honey samples with different methodologies produced a CV (%) <7%.

The comparative data obtained in this inter-laboratory study of the sugar profiling in honey samples shows that the glucose/fructose kit when applied to the RX misano system is a convenient and reliable analytical method and tool to facilitate the quality monitoring of honey.

Randox Food Diagnostics use the latest technology to make new developments in the analysis of quality within the honey industry. The RX misano is a user friendly machine, the assay kit included ready to use reagents and standard solution thus providing a convenient analytical tool to facilitate the quality control of honey in respect of compliance to legislative requirements.

Geomiel conducted this study in order to respond to feedback and demands from their customers to offer validated Glucose/ Fructose tests. Their lab had never previously conducted a study of this kind but have said that it enables them to "make a quality leap" and for Randox this validation using the RX misano showcases it's applicability to the honey industry. Geomiel state that "the experience with the RX misano is similar to the experience with the Evidence Investigator: reliability, speed and logical costs".

For more information on the RX misano contact [info@randoxfooddiagnostics.com](mailto:info@randoxfooddiagnostics.com)