

# Test Report: Commercial in Confidence

Fera Science Ltd,  
Sand Hutton,  
York,  
YO41 1LZ  
United Kingdom



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Test Report No.: FR002203\_S22034125

Date: 9<sup>th</sup> of August 2022

Customer:	The Scottish Bee Company
Analysis 1:	Biochemical profiling by <sup>1</sup> H-NMR
Analysis 2:	Metals analysis by ICP-MS
Analysis 3:	Analysis of 3-Phenyllactic acid (3-PLA) and 4-Hydroxyphenyllactic acid (4-HPLA) by LC-MS/MS
Analysis 4:	Multi-residue pesticide (including neonicotinoids) and veterinary medicine screen by LC-MS/MS
Analysis 5:	Addition of C4 sugars by EA-IRMS
Matrix:	Scottish Heather honey
Received:	23 <sup>rd</sup> May 2022
Analysed:	July to August 2022

## 1. SUMMARY

This report and the testing described within seeks to establish the authenticity of a sample of Scottish heather honey. Safety tests were also undertaken in relation to veterinary medicines and pesticides (including neonicotinoids).

A Nuclear Magnetic Resonance (NMR) spectrum of the sample extract was acquired and matched those of Scottish Heather honey held in the Fera database.

The trace element profile of the sample was broadly consistent with those for other honey types. However, the concentration of the nutritionally beneficial metal manganese (EFSA, 2013)<sup>1</sup> was found to be up to 10 times higher than a control honey (Manuka). Historic analytical data has found elevated levels of manganese in heather honey originating from Scotland.

Chemicals 3-Phenyllactic acid (3-PLA) and 4-Hydroxyphenyllactic acid (4-HPLA) are key markers of Scottish heather honey. The levels detected in the sample distinguish Scottish heather honey from most other honey types, except Manuka honey.\*

No residues of veterinary medicines or pesticides were found in the sample supplied at or above the maximum residue levels (MRL)<sup>2, 3</sup>.

\* Manuka honey has to be tested and shown to contain at least 20 mg/Kg of 3-PLA and 1 mg/Kg of 4-HPLA before it can be exported under the New Zealand Ministry for Primary Industry rules.

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## 2. BACKGROUND

This report describes the analytical testing of a Scottish heather honey provided by the Scottish Bee Company. The tests conducted at Fera were in addition to the routine testing for standard honey parameters. Fera have reviewed the CoA in relation to these tests and are satisfied that the sample provided met the required EU regulatory requirements for honey, this includes testing for relevant veterinary medicines.

## 3. SAMPLE DESCRIPTION

The sample was received at the laboratory in satisfactory condition and stored in a dark environment at ambient temperature prior to analysis.

The sample was in the manufacturers packaging with all seals intact.

A unique identifying number was assigned to the sample using the Fera laboratory information management system. The relevant sample details are shown in the table below.

Table 1. Sample information

Sample information				
Fera reference	Sample reference	Sample type	Batch/LOT code	Best before
S22-034125	The Scottish Bee Company	Scottish Heather Honey, 227 g	L21229	28/09/2025

## 4. SAMPLING AND ANALYSIS

### 4.1 Biochemical Profiling

The sample was accurately weighed ( $100 \text{ mg} \pm 10\text{mg}$ ) and dissolved into NMR buffer. The final concentration of honey was  $125 \text{ mg ml}^{-1}$ , the final buffer concentrations were 8.1 mM Trimethylsilylpropanoic acid (TSP, internal standard) and 125 mM phosphate buffer (pH = 6.5).

The  $^1\text{H}$  NMR experiment was carried out using a Bruker Avance 500 MHz NMR spectrometer equipped with a TCI cryoprobe. Data acquisition and processing were performed with the use of the software package Topspin v 1.3 (Bruker, Germany).

*This methodology does not fall under the scope of our ISO17025 accreditation.*

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## 4.2 Metals

Aliquots of homogenised test sample were digested in a mixture of nitric acid and hydrochloric acid using a high-pressure microwave system. Quantification was by inductively coupled plasma-mass spectrometry (ICP-MS) with collision cell. Quality checks included blanks, spikes and certified reference materials.

## 4.3 Targeted analysis of 3-Phenyllactic acid (3-PLA) and 4-Hydroxyphenyllactic acid (4-HPLA)

The concentrations of 3-PLA and 4-HPLA were determined by Liquid Chromatography with Tandem Mass Spectrometry detection (LC-MS/MS). Quantification was undertaken using a calibration series based on solvent standards. A reference honey and spiked samples were analysed alongside the test sample for quality assurance purposes.

## 4.4 Multi-residue pesticide (including neonicotinoids) and veterinary medicine screen

A sub-sample was extracted with acetonitrile in the presence of salts. Analysis was carried out using liquid chromatography with mass spectrometric detection (LC-MS/MS) in selected reaction monitoring mode. Spiked samples were analysed alongside the test sample to confirm the accuracy of the analysis.

## 4.5 C4 sugars

Analysis was carried out using an Elemental analyser coupled to an Isotope Ratio Mass spectrometer (EA-IRMS).

This analysis was performed by a third-party laboratory under accreditation to ISO17025.

**5. RESULTS**

**5.1 Biochemical Profiling**

NMR chemical shifts, peak intensity, *J*-couplings and lineshape were assessed against a database of known Scottish Heather honey spectra. The data from sample S22-034125 was found to be consistent with those previously recorded.

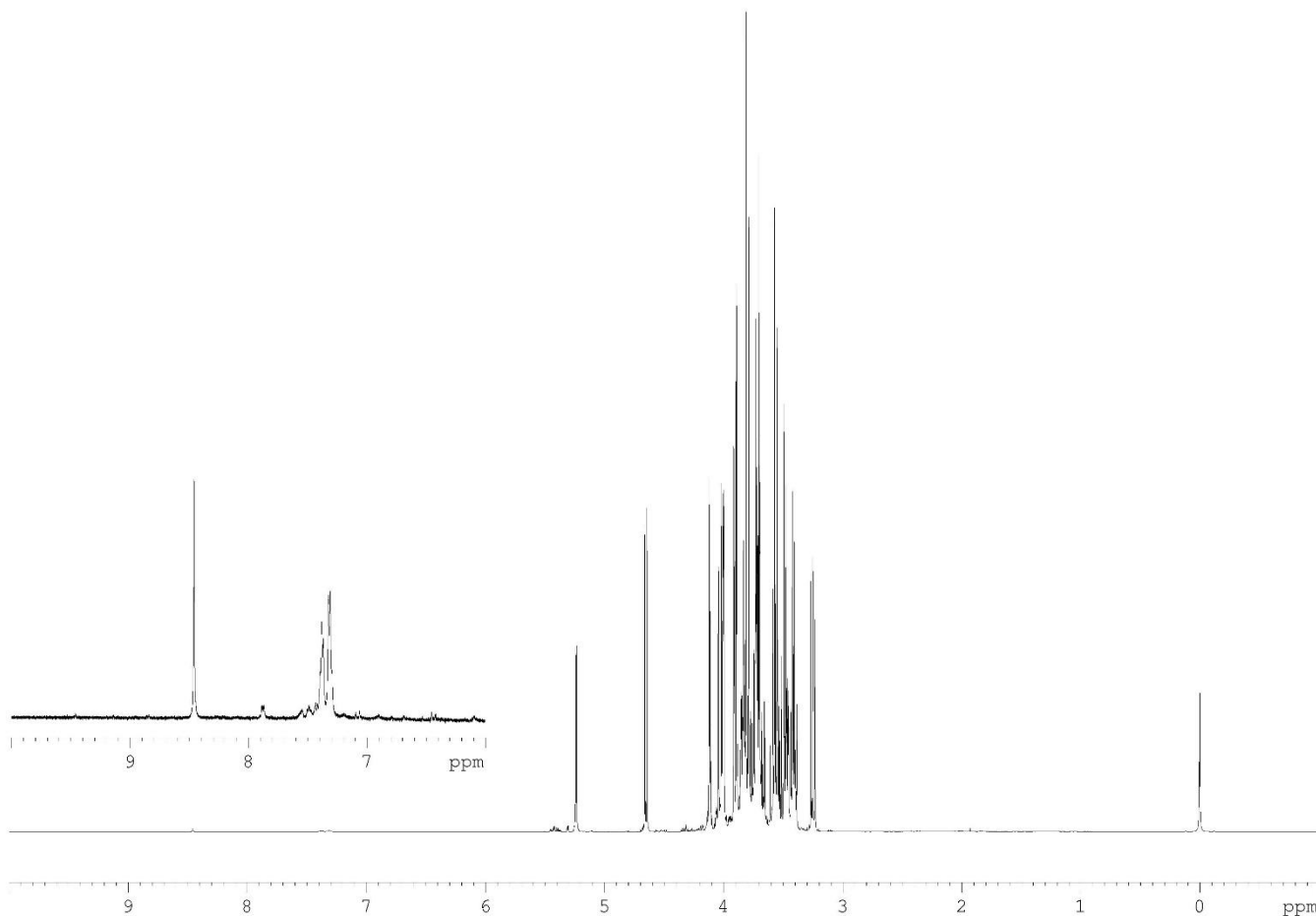


Figure 1. <sup>1</sup>H NMR data acquired from S22-034125

**5.2 Metals**

Table 2. Metals results

Metal	Sample result (mg/kg)
	S22-034125
Lithium	0.009
Beryllium	<0.001
Boron	9.00

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Metal	Sample result (mg/kg)
	S22-034125
Sodium	55.0
Magnesium	42.3
Aluminium	2.00
Phosphorus	60.0
Sulfur	80.0
Potassium	1970
Calcium	122
Scandium	<0.005
Titanium	<0.2
Vanadium	<0.01
Chromium	<0.05
Manganese	19.7
Iron	1.70
Cobalt	<0.005
Nickel	<0.07
Copper	0.540
Zinc	0.500
Gallium	<0.01
Germanium	<0.008
Arsenic	<0.005
Selenium	<0.01
Rubidium	7.95
Strontium	0.07
Yttrium	<0.001
Zirconium	<0.005
Niobium	<0.002
Molybdenum	<0.005
Ruthenium	<0.001
Rhodium	<0.005
Palladium	<0.001
Silver	<0.01
Cadmium	<0.005
Tin	<0.02
Antimony	<0.001
Tellurium	<0.005

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Metal	Sample result (mg/kg)
	S22-034125
Caesium	0.247
Barium	0.750
Lanthanum	<0.001
Cerium	0.012
Praseodymium	<0.001
Neodymium	<0.001
Samarium	<0.001
Europium	<0.001
Gadolinium	<0.001
Terbium	<0.001
Dysprosium	<0.001
Holmium	<0.001
Erbium	<0.001
Thulium	<0.001
Ytterbium	<0.001
Lutetium	<0.001
Hafnium	<0.001
Tantalum	<0.001
Tungsten	<0.002
Rhenium	<0.001
Osmium	<0.002
Iridium	<0.001
Platinum	<0.001
Gold	<0.005
Mercury	<0.005
Thallium	0.02
Lead	<0.005
Bismuth	<0.001
Thorium	0.001
Uranium	<0.001

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## 5.3 Targeted analysis of 3-Phenyllactic acid (3-PLA) and 4-Hydroxyphenyllactic acid (4-HPLA)

Table 3. 3-Phenyllactic acid and 4-Hydroxyphenyllactic acid results

Sample identification			Results (mg/kg)	
Fera reference	Sample reference	Batch/ LOT code	3-PLA	4-HPLA
S22-034125	The Scottish Bee Company, Scottish Heather Honey, 227 g	L21229	743.6	2.4

The presence of 3-PLA in honey is associated with originating from Scottish Heather<sup>4</sup>.

## 5.4 Multi-residue screen for pesticide and veterinary medicines

No residues were found in the sample supplied at or above the reporting limits quoted. The results relate only to the sample supplied.

The full set of results can be found in tables 5 and 6 in the Appendix.

## 5.5 C4 sugars

Table 4. C4 sugar results

Sample identification			Results			
Fera reference	Sample reference	Batch/ LOT code	Honey $\delta^{13}C$	Protein $\delta^{13}C$	Difference (Protein-Honey)	C4 Sugar Content**
			$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	%
S22-034125	The Scottish Bee Company, Scottish Heather Honey, 227 g	L21229	-27.35	-27.26	0.09	0.0

\*\* According to AOAC method 998.12 a result >7% indicates the addition of sugar derived from C4 plants.

## 6. CONCLUSION

The analytical test results strongly support that the tested sample is Scottish heather honey. Levels of pesticide (including neonicotinoids) and veterinary medicine residues are within the MRLs. Results also indicate that the sample did not contain sugar derived from C4 plants.

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## 7. REFERENCES

1. EFSA. *Scientific Opinion on Dietary Reference Values for manganese*. *EFSA Journal* 2013;11(11):3419
2. Source: EU & CODEX MRL Database (under Regulation 396/2005/EC)
3. [https://sitesv2.anses.fr/en/system/files/EURL\\_MMPR\\_guidance%20paper\\_final.pdf](https://sitesv2.anses.fr/en/system/files/EURL_MMPR_guidance%20paper_final.pdf)
4. Lorna Fyfe, Paulina Okoro, Euan Paterson, Shirley Coyle, Gordon J. McDougall. *Compositional analysis of Scottish honeys with antimicrobial activity against antibiotic-resistant bacteria reveals novel antimicrobial components*. *LWT - Food Science and Technology* 79 (2017) 52-59

<b>Issuing Officer:</b>	Mark Harrison, Analytical chemist	<b>Date:</b>	08/08/2022
<b>Countersigning Manager:</b>	Katharina Heinrich, Senior Scientist	<b>Date:</b>	09/08/2022

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## Appendix

Table 5. Multi-residue pesticide screen results

Pesticide	Concentration (mg/kg)
	S22-034125
abamectin	<0.01
acephate	<0.01
acetamiprid	<0.01
aldicarb	<0.01
aldicarb sulfone	<0.01
aldicarb sulfoxide	<0.01
allethrin	<0.01



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amitraz (sum)	<0.01
azamethiphos	<0.01
azinphos-ethyl	<0.01
azinphos-methyl	<0.01
BAC10	<0.05
BAC12	<0.05
BAC14	<0.05
BAC16	<0.05
bendiocarb	<0.01
bixafen	<0.01
boscalid	<0.01
cadusafos	<0.01
carbaryl	<0.01
carbendazim	<0.01
carbofuran	<0.001
carbofuran (3-hydroxy)	<0.001
chlorfenapyr	<0.01
chlorfenvinphos	<0.01
clothianidin	<0.01
coumaphos	<0.01
cyproconazole	<0.01
cyromazine	<0.01
DDAC	<0.05
dichlorvos	<0.01
diflubenzuron	<0.01
dimethoate	<0.01
dinotefuran	<0.01
DMF	<0.01
DMPF	<0.01
epoxiconazole	<0.01
etofenprox	<0.01
famoxadone	<0.01
fenoxy carb	<0.01
fenpropidin	<0.01
fenpropimorph	<0.01
fenthion	<0.01
fenthion sulfone	<0.01

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fenthion sulfoxide	<0.01
fipronil	<0.002
fipronil de-sulfinyl	<0.002
fipronil sulfone	<0.002
fluazifop (free acid)	<0.01
fluazifop-p-butyl	<0.01
flufenoxuron	<0.01
flumethrin	<0.01
fluquinconazole	<0.01
flusilazole	<0.01
haloxyfop (free acid)	<0.01
imidacloprid	<0.01
indoxacarb	<0.01
lufenuron	<0.01
malaoxon	<0.01
metaflumizone	<0.01
metazachlor	<0.01
methamidophos	<0.01
methiocarb	<0.01
methiocarb sulfone	<0.01
methiocarb sulfoxide	<0.01
methomyl	<0.01
mevinphos	<0.01
nitenpyram	<0.01
omethoate	<0.01
oxamyl	<0.01
phosmet	<0.01
phosphamidon	<0.01
phoxim	<0.01
pirimicarb	<0.01
pirimicarb-desmethyl	<0.01
prochloraz	<0.01
profenofos	<0.01
propetamphos	<0.01
propoxur	<0.01
prothioconazole-desthio	<0.01
pyrazophos	<0.01

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pyriproxyfen	<0.01
spinosad	<0.01
tebuconazole	<0.01
teflubenzuron	<0.01
tetraconazole	<0.01
thiacloprid	<0.01
thiamethoxam	<0.01
thiodicarb	<0.01
triazophos	<0.01

Table 6. Veterinary medicine residue results

	Result (µg/kg)
	S22-034125
<b>Aminoglycosides</b>	
Aparamycin	<1000
Dihydrostreptomycin	<20
Gentamycin	<50
Kanamycin	<100
Neomycin	<500
Paromomycin	<500
Spectinomycin	<200
Streptomycin	<20
<b>Amphenicols</b>	
Chloramphenicol	<0.15
Florfenicol	<10
Thiamphenicol	<10
<b>Beta-Lactams</b>	
Amoxicillin	<20
Ampicillin	<20
Cloxacillin	<20
Dicloxacillin	<20
Nafcillin	<20
Oxacillin	<20
Penicillin G	<20
Penicillin V	<20
<b>Cephalosporins</b>	
Cefalexin	<20 <sup>a</sup>
Cefalonium	<20 <sup>a</sup>
Cefapirin	<20 <sup>a</sup>
Cefazolin	<20 <sup>a</sup>
Cefoperazone	<20 <sup>a</sup>

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Cefquinome	<20 <sup>µ</sup>
Ceftiofur	<20 <sup>µ</sup>
Desacetyl-cephapirine	<20 <sup>µ</sup>
<b>Macrolides</b>	
3-O-Acetyltylosin	<2
Erythromycin	<2
Gamithromycin	<20
Josamycin	<5
Lincomycin	<2
Rifampicin	<5
Spiramycin	<20
Tilmicosin	<5
Tylosin	<2
Tylvalosin	<2
Virginiamycin	<10
<b>Nitrofurans</b>	
AHD	<0.5
AMOZ	<0.5
AOZ	<0.5
Semicarbazide	<0.5
<b>Quinolones</b>	
Danofloxacin	<10
Difloxacin	<10
Enrofloxacin	<10
Ciprofloxacin	<10
Flumequine	<10
Marbofloxacin	<10
Nalidixic acid	<10
Norfloxacin	<10
Oxolinic acid	<10
Sarafloxacin	<10
<b>Sulfonamides</b>	
Sulfachloropyridazine	<50
Sulfaclozine	<50 <sup>µ</sup>
Sulfadiazine	<50
Sulfadimethoxine	<50
Sulfadimidine/sulfamethazine	<50
Sulfadoxine	<50
Sulfamerazine	<50
Sulfamethizole	<50
Sulfamethoxyipyridazine	<50
Sulfamonomethoxine	<50
Sulfapyridine	<50

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Sulfaquinoxaline	<50
Sulfathiazole	<50
Sulfisoxazole	<50
Sulfaguanadine	<50
Sulfamethoxazole	<50
Sulfamoxole	<50
Sulfanilamide	<50
<b>Tetracyclines</b>	
Chlortetracycline	<10
epi-Chlortetracycline	<10
Doxycycline	<10
Oxytetracycline	<10
epi-Oxytetracycline	<10
Tetracycline	<10
epi-Tetracycline	<10

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