Novel Analytical Determination of Active Ingredient Concentration in Royal Jelly and Sucrose Diet Solutions

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Abstract

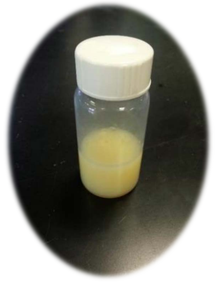
Pollinator risk assessments rely on information that begins with Tier 1 laboratory tests. These toxicity tests include: 48-hour adult acute contact and oral, 10-day oral adult chronic, acute (7-day) and chronic (22-day) larval studies. These tests require dosing via diets composed of either sucrose solutions or royal jelly. Depending on the physicochemical properties of the test material such as water solubility and hydrophobicity, these dose vehicles may be problematic to prepare and analyze. For materials with low toxicity, doses up to 100 µg a.i./bee are required. In many cases dose levels require solutions that vastly exceed the water solubility. The 50% sucrose solutions required for adult testing also impact the solubility. For some test materials, the properties of the royal jelly may aid the preparation of stable suspensions. For tests with poorly soluble materials, homogeneity is not only difficult to achieve, but also difficult to verify analytically. The development of robust analytical method validations to verify dosing concentrations can be challenging based on the properties of the material and the complex nature of the diets. We have developed methodology that allows the reproducible generation of homogeneous preparations with royal jelly and sucrose solutions for materials being tested above their water solubility. Analyses of the diets are performed using a variety of analytical techniques including LC/MS/MS, LC-UV, and GC/MS. Questions concerning complete consumption of diets that are dosed above solubility have led to additional analytical confirmation by analysis of larvae wells following completion of testing. Additional investigations are currently being performed to determine the functional solubility of poorly soluble materials in sucrose diet. In many cases the analytical methodology developed for the Tier 1 test can be leveraged for the analysis of higher tier matrices such as pollen, nectar, flowers, and honey.

The Problem – Chemical Solubility

Water solubility of <1930 mg/L limits testing to <100 µg/larva. For example a test substance with 1 mg/L solubility provides dose of 0.052 ug a.i./larva, lowering risk assessment value if no effect test results. Specialized mixing procedures are needed to provide homogeneity of test substance in sucrose and royal jelly diets.

Royal jelly is a natural emulsion and has many endogenous components that aid in the formation and stability of suspensions. These components include lipids, hydrocarbons, and proteins. Optimization of suspension homogeneity is an important first step. This ensures reproducible aliquots for dosing and analysis.

- **Typical Royal Jelly (wet weight)**
  - 60 to 70% moisture
  - 9 to18% proteins
  - 3 to 8% lipids
  - 6 to 18% hydrocarbons
  - 0.8 to 3.0% minerals
  - pH ~4.0



Typical Royal Jelly Procedure

1. Weigh out appropriate diet ‘type’ into an appropriate container.
2. Add acetone stock to the vial using an appropriate means.
3. Sonicate diet initially to maximize dissolution.
4. Mix on a magnetic stir plate for ≥ 30 minutes
5. Sonicate prior to feeding or analytical sampling.
6. Diet should stirring with a central vortex for chemistry sampling and/or feeding.

Sucrose Dosing Considerations

- Preparation of Dose Solutions

  50% sucrose by it’s nature has many properties which may further limit the solubility of the test material relative to pure water. When suspensions are generated settling may occur over time, changing the test material concentration over the course of the study. This can be particularly problematic during the dosing phase. Studies are needed to assess and confirm minimal concentration changes due to this phenomenon.

- Homogeneity

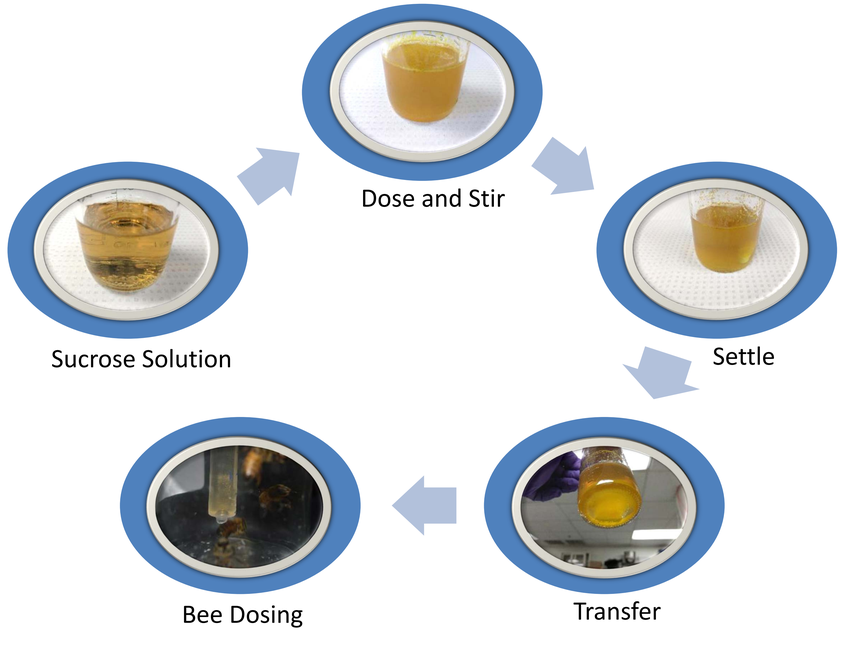
  All sucrose solutions have to be homogeneous without obvious signs of precipitation or settling throughout one feeding interval (about 24 hours).

- Stability

  Stability assesses both chemical stability as well as solution stability. Due to the formation of suspensions at the high concentrations needed, both settling and floating are a concern. Both are dependent on the test material and sucrose solution densities.

Preparation of Dosed Sucrose Diets

1. Add test material to 50% sucrose solution assuming a target dose of 100 µg/bee.
2. Sonicate to reduce particle size and optimize dissolution.
3. Mix on magnetic stir plate at room temperature.
4. Following mix, transfer diet to the incubator at test temperature and allow to settle for a static rest period.
5. Remove target volume of 50 mL as dose solution.
6. Use care to not disturb any layer of material that has settled or floated.



Royal Jelly Results by HPLC/MS/MS

Sample type	Fortified Conc. (µg/kg)	Royal Jelly Analytical Result Isomer a (µg/kg)	Analytical Result Isomer b (µg/kg)	Analytical Result Total (µg/kg)	Percent of Fortified
Acetone	0.00	<1.2500E+06	<8.7500E+04	< 1.3375E+06	NA
Acetone Stock	9,600,000	8.2571E+06	6.4609E+05	8.9032E+06	92.7%
Acetone Stock	20,000,000	1.6806E+07	1.3137E+06	1.8120E+07	90.6%
Acetone Stock	38,000,000	3.6215E+07	2.8344E+06	3.9050E+07	102.8%
Acetone Stock	77,000,000	7.0286E+07	5.4812E+06	7.5767E+07	98.4%
Acetone Stock	160,000,000	1.481E+08	1.1639E+07	1.5978E+08	99.9%
Control RJ	0.00	<2.2750E+04	<1.5925E+03	< 2.4343E+04	NA
Control RJ	0.00	<2.2700E+04	<1.5890E+03	< 2.4289E+04	NA
S. Control RJ	0.00	<2.2750E+04	<1.5925E+03	< 2.4343E+04	NA
S. Control RJ	0.00	<2.2750E+04	<1.5925E+03	< 2.4343E+04	NA
Royal Jelly	180,000	1.5338E+05	1.2081E+04	1.6546E+05	91.9%
Royal Jelly	180,000	1.5699E+05	1.2370E+04	1.6936E+05	94.1%
Royal Jelly	380,000	3.2881E+05	2.6227E+04	3.5503E+05	93.4%
Royal Jelly	380,000	3.2931E+05	2.5843E+04	3.5515E+05	93.5%
Royal Jelly	730,000	6.6948E+05	5.2722E+04	7.2220E+05	98.9%
Royal Jelly	730,000	6.8911E+05	5.5136E+04	7.4425E+05	102.0%
Royal Jelly	1,500,000	1.3183E+06	1.0448E+05	1.4227E+06	94.8%
Royal Jelly	1,500,000	1.3242E+06	1.0428E+05	1.4285E+06	95.2%
Royal Jelly	3,000,000	2.8565E+06	2.2576E+05	3.0823E+06	102.7%
Royal Jelly	3,000,000	2.7970E+06	2.2286E+05	3.0198E+06	100.7%
QC #1	100,000	8.8863E+04	7.0311E+03	9.5894E+04	95.9%
QC #2	750,000	6.9466E+05	5.4795E+04	7.4945E+05	99.9%
QC #3	3,000,000	2.6935E+06	2.1103E+05	2.9045E+06	96.8%

Sucrose Results by HPLC/MS/MS

Sample Analysis						
Sample I.D.	Sample type	Fortified Conc. mg/L	Analyte Response Area	Dilution Factor	Analytical Result mg/L	Percent of Fortified
21	50%	5000	13.13096	66700	1.5741E+03	31.5%
22	50%	5000	14.55683	66700	1.7427E+03	34.9%
23	67%	5000	4.27680	66700	5.2719E+02	10.5%
24	67%	5000	4.60201	66700	5.6564E+02	11.3%
26	QC 1	1000	9.00619	66700	1.0864E+03	108.6%
27	QC 2	2500	22.01436	66700	2.6245E+03	105.0%
28	QC 3	5000	39.12722	66700	4.6479E+03	93.0%

Dose Verification - Analytical Method Considerations

- Analytical Precision

The precision of the analytical method is reported as repeatability of recovery at each fortification level. Five determinations are made at each fortification level for each validation. The precision is calculated for the fortified samples in terms of the standard deviation (SD), overall relative standard deviation (RSD), and/or coefficient of variation (CV). RSD values ≤ 20% for each fortification level is considered acceptable, while values ≤10% is considered ideal.

- Analytical Recovery

The accuracy of the method is reported as the mean recovery ± the relative standard deviation based on representative samples prepared by fortification of the control matrix with a known quantity of the analyte. The fortification range encompasses the LOQ and the predicted highest level to be used during testing. The samples described above will be prepared ‘de novo’. Mean recoveries of 70-110% for each fortification level will be considered acceptable. During testing, individual quality control sample recovery acceptability will be set at 80-120%.