**SUPPLEMENTARY MATERIAL to paper entitled:**

**Method development and validation for low-level propineb and propylenethiourea analysis in baby food, infant formula, and related matrices using liquid chromatography-tandem mass spectrometry**

Lukas Vaclavika\*, Jeffrey J. Shipparb, Urairat Koesukwiwatc, and Katerina Mastovskab

aEurofins Food Integrity and Innovation, Otley Road, Harrogate North Yorkshire, HG3 1PY, UK

bEurofins Food Integrity and Innovation, 3301 Kinsman Blvd., Madison, WI 53704, USA

cEurofins Food Integrity and Innovation, The Synergy, International Business Park, Singapore

\* Corresponding author: Lukas Vaclavik (LukasVaclavik@eurofins.co.uk

Phone: +44 (0)1423848583

**This document provides more detailed information to the paper mentioned above.**

**The following information is included:**

**Table S1** Matrix effects obtained for propylenebisdithiocarbamate-dimethyl (PBDC-dimethyl) and propylenethiourea (PTU)

**Table S2** Trueness (recovery) and precision (RSD) data obtained for propineb

**Table S3** Trueness (recovery) and precision (RSD) data obtained for propylenethiourea

**Table S4** Robustness testing results

**Figure S1** LC–ESI-MS/MS extracted ion chromatograms of PBDC-methyl quantification MRM (*m/z* 255.1>207.1) and all MRMs (an overlay of all 6 MRMs normalized to the quantitation MRM) obtained in the analysis of propineb in blank and LOQ-spiked milk-based infant formula powder (A, D), soy protein isolate (B, E) and cereal-based baby food (C, F) samples

**Figure S2** LC–APCI-MS/MS extracted ion chromatograms of PTU quantification MRM (*m/z* 117.1>58.2) and all MRMs (an overlay of all 3 MRMs normalized to the quantitation MRM) obtained in the analysis of PTU in selected blank and LOQ-spiked milk-based infant formula powder (A, D), soy protein isolate (B, E) and cereal-based baby food (C, F) samples, including extracted ion chromatograms of the internal standard PTU-*d*6 (*m/z* 123.1>64.2) in the given matrix spikes

**Table S1** Matrix effects obtained for propylenebisdithiocarbamate-dimethyl (PBDC-dimethyl) and propylenethiourea (PTU)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Matrix** | **PBDC-dimethyl** | | | | **PTU** | | | |
| **Analyte concentration (ng mL–1)** | **Peak area** | | **Matrix effect (%)** | **Analyte/internal standard concentration (ng mL–1)** | **Analyte-to-internal standard peak area ratio** | | **Matrix effect (%)** |
| **Solvent** | **Matrix** | **Solvent** | **Matrix** |
| Powdered infant formula | 0.075 | 12568 | 12379 | 98.5 | 0.15/5 | 0.0168 | 0.0162 | 96.0 |
| 0.3 | 57045 | 53276 | 93.4 | 1.0/5 | 0.1266 | 0.1227 | 96.9 |
| 1.5 | 293696 | 283201 | 96.4 | 5.0/5 | 0.6692 | 0.6528 | 97.5 |
| Ready to feed infant formula | 0.075 | 13469 | 13465 | 100 | 0.15/5 | 0.0168 | 0.0163 | 96.7 |
| 0.3 | 56931 | 57778 | 101 | 1.0/5 | 0.1266 | 0.1216 | 96.0 |
| 1.5 | 302345 | 301779 | 99.8 | 5.0/5 | 0.6692 | 0.6316 | 94.4 |
| Maltodextrin | 0.075 | 12216 | 12186 | 99.8 | 0.15/5 | 0.0168 | 0.0163 | 96.6 |
| 0.3 | 52101 | 54806 | 105 | 1.0/5 | 0.1266 | 0.1235 | 97.6 |
| 1.5 | 290381 | 285759 | 98.4 | 5.0/5 | 0.6692 | 0.6525 | 97.5 |
| Soy protein isolate | 0.075 | 13072 | 12078 | 92.4 | 0.15/5 | 0.0168 | 0.0160 | 95.1 |
| 0.3 | 58798 | 50418 | 85.7 | 1.0/5 | 0.1266 | 0.1224 | 96.7 |
| 1.5 | 293291 | 274634 | 93.6 | 5.0/5 | 0.6692 | 0.6394 | 95.5 |
| Soybean oil | 0.075 | 13281 | 10972 | 82.6 | 0.15/5 | 0.0168 | 0.0164 | 97.6 |
| 0.3 | 58682 | 46521 | 79.3 | 1.0/5 | 0.1266 | 0.1266 | 100 |
| 1.5 | 320917 | 246020 | 76.7 | 5.0/5 | 0.6692 | 0.6273 | 93.7 |
| Soy lecithin | 0.075 | 13469 | 14614 | 109 | 0.15/5 | 0.0168 | 0.0164 | 97.4 |
| 0.3 | 57635 | 54730 | 95.0 | 1.0/5 | 0.1266 | 0.1229 | 97.1 |
| 1.5 | 309411 | 299873 | 96.9 | 5.0/5 | 0.6692 | 0.6275 | 93.8 |
| Fruit-based baby food | 0.075 | 13348 | 12922 | 96.8 | 0.15/5 | 0.0168 | 0.0157 | 93.0 |
| 0.3 | 56545 | 56339 | 99.6 | 1.0/5 | 0.1266 | 0.1256 | 99.2 |
| 1.5 | 306045 | 294341 | 96.2 | 5.0/5 | 0.6692 | 0.6679 | 99.8 |
| Cereal-based baby food | 0.075 | 12467 | 13527 | 109 | 0.15/5 | 0.0168 | 0.0168 | 99.5 |
| 0.3 | 53608 | 58390 | 109 | 1.0/5 | 0.1266 | 0.1282 | 101 |
| 1.5 | 285573 | 296740 | 104 | 5.0/5 | 0.6692 | 0.6679 | 99.8 |

**Table S2** Trueness (recovery) and precision (RSD) data obtained for propineb

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Matrix** | **Spiking level (mg kg–1)** | **Solvent standard calibration** | | | **Matrix-matched standard calibration** | | |
| **Recovery (%)** | **Mean recovery (%)** | **RSD (%)** | **Recovery (%)** | **Mean recovery (%)** | **RSD (%)** |
| Powdered infant formula | 0.003  (Day 1) | 106 | 109 | 4.1 | 110 | 113 | 3.3 |
| 104 | 109 |
| 110 | 114 |
| 112 | 115 |
| 115 | 118 |
| 0.003  (Day 2) | 116 | 113 | 3.5 | n/a | n/a | n/a |
| 116 | n/a |
| 110 | n/a |
| 114 | n/a |
| 107 | n/a |
| 0.01  (Day 1) | 118 | 115 | 2.2 | 120 | 116 | 3.9 |
| 111 | 120 |
| 115 | 113 |
| 114 | 118 |
| 115 | 110 |
| 0.01  (Day 2) | 111 | 109 | 1.4 | n/a | n/a | n/a |
| 109 | n/a |
| 110 | n/a |
| 107 | n/a |
| 109 | n/a |
| Ready to feed infant formula | 0.003 | 118 | 116 | 2.2 | 119 | 118 | 1.5 |
| 114 | 116 |
| 120 | 120 |
| 115 | 117 |
| 115 | 116 |
| 0.01 | 119 | 120 | 1.5 | 119 | 119 | 1.8 |
| 122 | **122** |
| 120 | 119 |
| 120 | 120 |
| 117 | 116 |
| Maltodextrin | 0.003 | 118 | 113 | 4.6 | 119 | 114 | 4.6 |
| 106 | 107 |
| 118 | 119 |
| 110 | 111 |
| 114 | 115 |
| 0.01 | 106 | 115 | 4.7 | 106 | 114 | 4.3 |
| 117 | 116 |
| 120 | 118 |
| 113 | 113 |
| 117 | 117 |
| Soy protein isolate | 0.003 | 98.8 | 99.0 | 1.4 | 111 | 111 | 1.3 |
| 101 | 113 |
| 97.2 | 109 |
| 99.2 | 111 |
| 98.6 | 110 |
| 0.01 | 106 | 104 | 2.3 | 116 | 114 | 2.4 |
| 103 | 113 |
| 101 | 110 |
| 107 | 117 |
| 105 | 115 |

n/a: Quantification based on matrix-matched standards was not performed on day 2.

**Table S2** Continued

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Matrix** | **Spiking level (mg kg–1)** | **Solvent standard calibration** | | | **Matrix-matched standard calibration** | | |
| **Recovery (%)** | **Mean recovery (%)** | **RSD (%)** | **Recovery (%)** | **Mean recovery (%)** | **RSD (%)** |
| Soybean oil | 0.003 | 118 | 113 | 4.6 | 119 | 114 | 4.6 |
| 106 | 107 |
| 118 | 119 |
| 110 | 111 |
| 114 | 115 |
| 0.01 | 106 | 115 | 4.7 | 106 | 114 | 4.3 |
| 117 | 116 |
| 120 | 118 |
| 113 | 113 |
| 117 | 117 |
| Soy lecithin | 0.003 | 96.4 | 98.9 | 2.1 | 93.0 | 97.2 | 3.9 |
| 101 | 103 |
| 101 | 98.2 |
| 97.9 | 97.1 |
| 98.2 | 94.9 |
| 0.01 | 78.7 | 85.5 | 12 | 79.2 | 86.1 | 12.8 |
| 74.6 | 74 |
| 92.7 | 93.7 |
| 82.1 | 82.7 |
| 99.4 | 101 |
| Fruit-based baby food | 0.003 | 110 | 113 | 2.1 | 111 | 114 | 2.1 |
| 115 | 116 |
| 110 | 111 |
| 114 | 115 |
| 114 | 115 |
| 0.01 | 118 | 116 | 1.1 | **121** | 119 | 1.0 |
| 116 | 119 |
| 116 | 120 |
| 115 | 118 |
| 115 | 119 |
| Cereal-based baby food | 0.003 | 110 | 110 | 2.4 | 107 | 105 | 6.8 |
| 109 | 106 |
| 111 | 109 |
| 114 | 110 |
| 107 | 92.5 |
| 0.01 | 96.1 | 99.1 | 9.3 | 94.2 | 97.4 | 9.1 |
| 91.6 | 91.0 |
| 94.8 | 93.0 |
| 97.9 | 96.0 |
| 115 | 113 |

**Table S3.** Trueness (recovery) and precision (RSD) data obtained for propylenethiourea.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Matrix** | **Spiking level (mg kg–1)** | **Recovery (%)** | **Mean recovery (%)** | **RSD (%)** |
| Powdered infant formula | 0.003  (Day 1) | 114 | 109 | 3.6 |
| 110 |
| 105 |
| 111 |
| 105 |
| 0.003  (Day 2) | 108 | 106 | 1.3 |
| 105 |
| 105 |
| 105 |
| 107 |
| 0.01  (Day 1) | 88.8 | 95.0 | 4.4 |
| 98.2 |
| 99.5 |
| 93.8 |
| 94.7 |
| 0.01  (Day 2) | 95.3 | 95.0 | 1.4 |
| 94.1 |
| 95.0 |
| 97.1 |
| 93.7 |
| Ready to feed infant formula | 0.003 | 113 | 115 | 2.2 |
| 115 |
| 113 |
| 119 |
| 114 |
| 0.01 | 98.0 | 96.2 | 3.3 |
| 93.0 |
| 99.1 |
| 92.6 |
| 98.3 |
| Maltodextrin | 0.003 | 105 | 108 | 4.4 |
| 113 |
| 101 |
| 110 |
| 110 |
| 0.01 | 95.4 | 91.3 | 2.8 |
| 88.6 |
| 90.3 |
| 90.6 |
| 91.5 |
| Soy protein isolate | 0.003 | 82.3 | 88.0 | 4.4 |
| 92.6 |
| 90.5 |
| 87.7 |
| 87.0 |
| 0.05 | 94.3 | 96.4 | 4.7 |
| 89.7 |
| 101 |
| 97.1 |
| 99.9 |

**Table S3.** Continued.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Matrix** | **Spiking level (mg kg–1)** | **Recovery (%)** | **Mean recovery (%)** | **RSD (%)** |
| Soybean oil | 0.01 | 96.6 | 98.8 | 2.2 |
| 99.3 |
| 102 |
| 97.1 |
| 99.1 |
| 0.05 | 109 | 110 | 3.5 |
| 108 |
| 106 |
| 116 |
| 110 |
| Soy lecithin | 0.003 | 103 | 101 | 7.3 |
| 99.7 |
| 111 |
| 90.3 |
| 101 |
| 0.01 | 88.1 | 89.0 | 3.4 |
| 90.9 |
| 91.2 |
| 84.1 |
| 90.6 |
| Fruit-based baby food | 0.003 | 90.7 | 89.7 | 3.7 |
| 92.2 |
| 89.2 |
| 92.2 |
| 84.3 |
| 0.01 | 93.2 | 94.4 | 3.6 |
| 96.3 |
| 98.7 |
| 94.0 |
| 89.6 |
| Cereal-based baby food | 0.003 | 95.3 | 99.8 | 6.1 |
| 92.7 |
| 107 |
| 105 |
| 99.2 |
| 0.01 | 93.7 | 101 | 6.8 |
| 104 |
| 92.9 |
| 104 |
| 108 |

**Table S4.** Robustness testing results.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Propineb analysis** | | | | | |
| **Replicate** | **Recovery (%)** | | | | |
| **Original parameters** | **Extraction time** | | **Dimethyl sulphate concentration** | |
| **20 min** | **40 min** | **0.025 M** | **0.100 M** |
| 1 | 111 | 110 | 113 | 106 | 116 |
| 2 | 108 | 109 | 112 | 111 | 118 |
| 3 | 106 | 110 | 111 | 110 | 116 |
| Mean (%) | 108 | 110 | 112 | 109 | 117 |
| RSD (%) | 2.2 | 0.6 | 1.1 | 2.2 | 1.0 |
| Relative percent difference (%) | n/a | 1.2 | 3.2 | 0.2 | 7.5 |
| **PTU analysis** | | | | | |
| **Replicate** | **Recovery (%)** | | | | |
| **Original parameters** | **Extraction time** | | **Volume of crude extract loaded on SPE** | |
| **5 min** | **15 min** | **0.5 mL** | **1.5 mL** |
| 1 | 99.5 | 98.7 | 98.3 | 102 | 107 |
| 2 | 99.6 | 103 | 99.1 | 101 | 93.7 |
| 3 | 102 | 94.0 | 93.7 | 98.6 | 100 |
| Mean (%) | 100 | 99.0 | 97.0 | 101 | 100 |
| RSD (%) | 1.5 | 4.6 | 3.0 | 1.7 | 6.6 |
| Relative percent difference (%) | n/a | -1.8 | -3.4 | 0.1 | -0.1 |

n/a: Not applicable.



**Figure S1.**



**Figure S2.**