

Supplementary Materials: Development and Validation of a UHPLC-ESI-MS/MS Method for Quantification of Oleandrin and Other Cardiac Glycosides and Evaluation of Their Levels in Herbs and Spices from the Belgian Market

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Table S1. Comparison of performance characteristics of different LC-MS/MS methods for analysis of cardiac glycosides.

| Cardiac Glycoside(s) | Matrix | Method | Limit of Quantification | Recovery (%) ³ | Repeatability (%) | Reproducibility (%) | Reference ⁵ |
|----------------------|-------------------|-------------|--------------------------|---------------------------|-------------------|---------------------|------------------------|
| Oleandrin | culinary herbs | UHPLC-MS/MS | 1.5 ng/g | 83 | 6 | 8 | current study |
| Digoxin | | | 7.5 ng/g | 115 | 9 | 9 | |
| Digitoxin | | | 15 ng/g | 92 | 10 | 14 | |
| Convallatoxin | | | 7.5 ng/g | 55 | 9 | 14 | |
| Oleandrin | human urine | UHPLC-MS/MS | 0.025 ng/mL | 84 | 3 | 13 | current study |
| Digoxin | | | 0.5 ng/mL | 92 | 3 | 8 | |
| Digitoxin | | | 0.25 ng/mL | 89 | 5 | 12 | |
| Convallatoxin | | | 1 ng/mL | 94 | 3 | 10 | |
| Ouabain | | | 1 ng/mL | 90 | 5 | 13 | |
| Digoxin | human serum | HPLC-MS/MS | 0.12 ng/mL | 96 | 6 | 9 | [1] |
| Digitoxin | | | 0.4 ng/mL | 91 | 11 | 14 | |
| Digoxin | human whole blood | HPLC-MS/MS | 0.15 ng/mL ¹ | 106 | 7 | | [2] |
| Digitoxin | | | 0.3 ng/mL ¹ | 113 | 10 | / | |
| Digoxin | human urine | | 0.15 ng/mL ¹ | 94 | 5 | | |
| Digitoxin | | | 0.3 ng/mL ¹ | 89 | 9 | | |
| Oleandrin | bovine serum | UHPLC-MS/MS | 0.36 ng/mL | 63 | | | [3] |
| | bovine blood | | | 78 | / | / | |
| | bovine heart | | | 80 | / | / | |
| | bovine liver | | | 80 | | | |
| Oleandrin | human plasma | HPLC-MS/MS | 3 ng/mL ¹ | / ⁴ | / | / | [4] |
| Oleandrin | human whole blood | HPLC-MS/MS | 0.023 ng/mL ² | >92 | <15 | <15 | [5] |

| Cardiac Glycoside(s) | Matrix | Method | Limit of Quantification | Recovery (%) ³ | Repeatability (%) | Reproducibility (%) | Reference ⁵ |
|----------------------|-------------------|-------------|-------------------------|---------------------------|-------------------|---------------------|------------------------|
| Oleandrin | herbal cosmetics | UHPLC-MS/MS | 5 ng/g | 98 | 5 | 5 | [6] |
| Digoxin | culture medium | HPLC-MS/MS | 0.5 ng/mL | 99 | 5 | 5 | [7] |
| Ouabain | | | | 101 | 3 | 4 | |
| Digoxin | cell lysate | HPLC-MS/MS | 0.5 ng/mL | 101 | 8 | 7 | [7] |
| Ouabain | | | | 105 | 4 | 7 | |
| Digoxin | human serum | HPLC-MS/MS | 0.1 ng/mL | 101 | 6 | 8 | [8] |
| Oleandrin | human blood | LC-MS/MS | 3 ng/mL ¹ | 98 | <11 | <11 | [9] |
| | human liver | | 6 ng/g ¹ | 112 | | | |
| Digoxin | human whole blood | UHPLC-MS/MS | 0.28 ng/mL | 104 | 6 | 6 | [10] |
| Convallatoxin | human whole blood | UHPLC-MS/MS | 4.2 ng/mL ¹ | 111 | 13 | 13 | [11] |
| Digitoxin | | | 0.3 ng/mL ¹ | 102 | 14 | 14 | |
| Digoxin | | | 0.6 ng/mL ¹ | 98 | 7 | 9 | |
| Oleandrin | | | 0.3 ng/mL ¹ | 96 | 11 | 15 | |

¹ estimated from the reported limits of detection using a factor of 3; ² estimated from the lowest reported value with regards to S/N of 10; ³ a mean value across different validated levels, where applicable; ⁴ not reported or not evaluated; ⁵ a list of references is provided at the end of Supplementary Materials.

Table S2. Overview of culinary herbs and spices collected in Belgian food stores.

| Sample Code | Product Name | Farming | Country of Production | Ingredients |
|-------------|------------------------|-------------|-----------------------|--|
| Sample 1 | Garam masala spice mix | non-organic | Germany | coriander (30%), black pepper (11%), cumin, garlic (10%), chili pepper (5.5%), cinnamon (4%), clove (3.5%), ginger, bay leaf (3%) |
| Sample 2 | Italian herb mix | organic | n.s. ¹ | mild chili, oregano, thyme, bay leaf, sage, ground coriander seeds, basil, black pepper |
| Sample 3 | Mix for pasta | organic | n.s. | wild marjoram, thyme, basil, rosemary, garlic, onion, savory, bay leaf |
| Sample 4 | Herb mix for fish | organic | n.s. | fennel, thyme, parsley, bay leaf |
| Sample 5 | Bay leaves | organic | Turkey | bay leaves |
| Sample 6 | Garam masala spice mix | non-organic | France | coriander seeds (49%), black pepper, Jamaican pepper, cumin (6%), cardamom (6%), turmeric, nutmeg, ginger, clove, bay leaf, cinnamon, chili powder, cayenne pepper |

| Sample Code | Product Name | Farming | Country of Production | Ingredients |
|-------------|-----------------------------|-------------|-----------------------|--|
| Sample 7 | Mix for beef stew | non-organic | France | dried onion (36%), bell pepper (6%), coriander seed, mustard seed (4%), Jamaican pepper, ginger, white pepper, nutmeg, bay leaf, thyme |
| Sample 8 | Taste refiner | non-organic | n.s. | onion, garlic, turmeric, celery seed, clove, bay leaf |
| Sample 9 | Bouquet garni | non-organic | n.s. | bay leaf, thyme |
| Sample 10 | Bay leaves | non-organic | n.s. | bay leaves |
| Sample 11 | Mix for mussels | non-organic | n.s. | vegetables, chives, parsley, tarragon, dill, bay leaf, thyme, pepper, mustard, fennel, turmeric |
| Sample 12 | Bay leaves | non-organic | n.s. | bay leaves |
| Sample 13 | Bay leaves | non-organic | The Netherlands | bay leaves |
| Sample 14 | Garam masala spice mix | non-organic | n.s. | coriander seeds, cumin seeds (22%), bell pepper, black pepper, cinnamon, coriander leaves, cardamom (4%), bay leaves, cloves, cayenne pepper |
| Sample 15 | Italian herb mix | non-organic | n.s. | garlic, onion, bell pepper, oregano, basil, bay leaf, pepper, nutmeg |
| Sample 16 | Bay leaves | organic | n.s. | bay leaves |
| Sample 17 | Herb mix for fish | organic | n.s. | onion, dill, black pepper, tarragon, bay leaf, fennel seeds, thyme |
| Sample 18 | Bay leaves | organic | Turkey | bay leaves |
| Sample 19 | Ras el hanout spice mix | organic | n.s. | turmeric, cinnamon, fennel, clove, nutmeg, black pepper, ginger, bay leaf, cayenne pepper |
| Sample 20 | Chimichurri dry mix | organic | n.s. | bell pepper, onion, tomato, basil, oregano, thyme, black pepper, seaweed, garlic, cumin seed, bay leaf, cayenne pepper |
| Sample 21 | Vadouvan spice mix | organic | n.s. | onion, turmeric, cardamom, garlic, fennel seed, cumin seed, seaweed, mustard seed, carrot, tarragon, clove, fenugreek seed, cinnamon, bay leaf, cayenne pepper, lovage |
| Sample 22 | Italian herb mix | organic | n.s. | basil, marjoram, rosemary, onion, thyme, seaweed, savory, garlic, bay leaf, bell pepper |
| Sample 23 | Roasted vegetables herb mix | non-organic | n.s. | onion, garlic, rosemary, tomato, parsley, thyme, black pepper, bay leaf |
| Sample 24 | Spice mix | non-organic | Sweden | black pepper, juniper (16%), roasted garlic, mushroom (5.5%), parsley, rosemary, thyme, carrot, pink pepper, coriander, bay leaf |
| Sample 25 | Mix for beef stew | non-organic | n.s. | black pepper, mustard seed, nutmeg, onion, garlic, thyme, bay leaf |

| Sample Code | Product Name | Farming | Country of Production | Ingredients |
|-------------|--------------------------------------|-------------|-----------------------|---|
| Sample 26 | Curry mix | non-organic | n.s. | coriander seed, turmeric, fenugreek, mustard seed, cumin seed, cinnamon, bay leaf, clove, garlic |
| Sample 27 | Mix for chicken tikka masala | non-organic | n.s. | bell pepper, lemongrass, turmeric, cayenne pepper, coriander seeds, nutmeg, cumin seeds, fennel seeds, white pepper, garlic, onion, chives, bay leaves, thyme |
| Sample 28 | Mix for beef stew | non-organic | n.s. | black pepper (15%), mustard seeds, nutmeg (5%), onion, thyme (5%), bay leaf (4%), garlic (5%) |
| Sample 29 | Tajine spice blend | non-organic | n.s. | ginger, cumin (4%), cinnamon (3%), paprika, allspice, bay leaf, cardamom (1%), black pepper, carrot, onion, leek, parsnip, garlic, oregano, basil, cabbage, paprika |
| Sample 30 | Mix for spaghetti | non-organic | n.s. | vegetables (39.9%), garlic, oregano, thyme, bay leaf, rosemary, pepper, parsley root |
| Sample 31 | Mix for beef stew | non-organic | n.s. | onion (15%), tomato (11%), pepper, clove, parsley root, bay leaf |
| Sample 32 | Bay leaves (fresh) | non-organic | Morocco | bay leaves |
| Sample 33 | Bouquet garni | non-organic | n.s. | thyme, bay leaf |
| Sample 34 | Bay leaves | non-organic | n.s. | bay leaves |
| Sample 35 | Mix for couscous | non-organic | n.s. | red pepper, cumin, coriander seeds, pepper, dried onion, white pepper, bay leaf, clove |
| Sample 36 | Bouquet garni for fish | non-organic | n.s. | thyme, coriander, fennel, basil, parsley, bay leaf |
| Sample 37 | Bouquet garni for meat | non-organic | n.s. | thyme, coriander, marjoram, parsley, bay leaf |
| Sample 38 | Bay leaves | non-organic | n.s. | bay leaves |
| Sample 39 | Bay leaves | non-organic | Turkey | bay leaves |
| Sample 40 | Bay leaves (crushed) | non-organic | n.s. | bay leaves |
| Sample 41 | Mix for paella | non-organic | n.s. | dried garlic, red pepper (18%), mild pepper (17%), dried onion (14%), turmeric, bay leaf, savory, black pepper, clove |
| Sample 42 | Bouquet garni for fish and shellfish | non-organic | EU | dried carrot (31.6%), savory, dried onion (10.5%), dried garlic, coriander seeds, black pepper, Jamaican pepper, basil, bay leaf (5.3%), fennel |
| Sample 43 | Bouquet garni for meat and stew | non-organic | EU | savory, bay leaf (27.7%), rosemary, basil (8.2%), black pepper, thyme (4%) |

| Sample Code | Product Name | Farming | Country of Production | Ingredients |
|-------------|-------------------------|-------------|-----------------------|---|
| Sample 44 | Mix for chicken | non-organic | EU | bell pepper (13%), turmeric (4%), coriander seeds (3%), onion powder, garlic powder, parsley, black pepper, thyme, ginger, bay leaf, nutmeg, cayenne pepper, cumin |
| Sample 45 | Bay leaves (crushed) | non-organic | n.s. | bay leaves |
| Sample 46 | Bay leaves (crushed) | non-organic | n.s. | bay leaves |
| Sample 47 | Mix for fish | non-organic | n.s. | fennel, garlic, coriander, cumin, bay leaf, pepper, mustard, clove, Mexico pepper, fenugreek |
| Sample 48 | Mix for chicken | non-organic | n.s. | bell pepper, coriander, turmeric, mustard seeds, cumin, chilies, fennel seeds, fenugreek, cardamom, black pepper, garlic, cloves, garlic, nutmeg, onion, chervil, chives, thyme, bay leaf |
| Sample 49 | Bay leaves | organic | n.s. | bay leaves |
| Sample 50 | Mix for couscous | organic | n.s. | bell pepper, coriander (22%), cumin (20%), red pepper, onion (9%), chili, bay leaf, black pepper, clove |
| Sample 51 | Mix for paella | organic | n.s. | turmeric, dried onion, red bell pepper (14%), bell pepper, dried garlic (10%), fennel, black pepper, chili, Jamaican pepper, parsley, bay leaf (3%) |
| Sample 52 | Bouquet garni | non-organic | n.s. | parsley, thyme, bay leaf |
| Sample 53 | Bouquet garni | organic | n.s. | thyme, bay leaf, rosemary |
| Sample 54 | Mix for minced meat | non-organic | n.s. | garlic, onion, paprika, oregano, basil, bay leaf, pepper, nutmeg |
| Sample 55 | Mediterranean spices | non-organic | n.s. | rosemary (20%), thyme (5,9%), tomato (12%), onion, garlic, parsley, black pepper, bay leaf |
| Sample 56 | Mix for mussels | non-organic | n.s. | onion, carrot, leek, tomato, parsley, thyme, celery leaves, bay leaves |
| Sample 57 | Mix for pizza | non-organic | n.s. | oregano, cayenne pepper, red pepper, bay leaf, paprika, garlic, basil, thyme |
| Sample 58 | Mix for fish | non-organic | n.s. | dill, fennel, bay leaf, parsley |
| Sample 59 | Ras el hanout spice mix | non-organic | n.s. | turmeric, caraway, coriander, cumin, chili pepper, ginger, fenugreek, cinnamon, fennel, bay leaf, cloves, pepper |
| Sample 60 | Mix for tandoori | non-organic | n.s. | bell pepper, garlic, pepper, coriander, cumin, caraway seeds, rosemary, thyme, cloves, bay leaves, cinnamon, black pepper |
| Sample 61 | Herbes de Provence | non-organic | n.s. | bay leaf, rosemary, savory, oregano, thyme |

| Sample Code | Product Name | Farming | Country of Production | Ingredients |
|-------------|-----------------|-------------|-----------------------|---|
| Sample 62 | Mix for mussels | non-organic | n.s. | chives, parsley, tarragon, dill, bay leaf, thyme, pepper, mustard, fennel, turmeric, vegetables |
| Sample 63 | Mix for grill | non-organic | n.s. | marjoram, basil, sage, onion, bay leaf, rosemary |
| Sample 64 | Mix for pizza | non-organic | n.s. | oregano, chili, pepper, bay leaf, powdered paprika, garlic, basil, thyme |
| Sample 65 | Bouquet garni | non-organic | n.s. | thyme, bay leaf, parsley |

¹n.s.: not specified.

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