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World Health Organization

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**Agenda Item 3** **CRD06**

**ORIGINAL LANGUAGE ONLY**

**JOINT FAO/WHO FOOD STANDARDS PROGRAMME**

**FAO/WHO COORDINATING COMMITTEE FOR NORTH AMERICA AND THE SOUTH WEST PACIFIC**

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# UPDATE ON THE METHODS OF ANALYSIS OF SCOPOLETIN IN NONI FRUIT JUICE

*(Submitted by Samoa, with the support of New Zealand, Australia and the International Union of Food Science and Technology - IUFoST[1])*

## Background

1. CAC46 (2023) adopted the *Regional Standard for Fermented Noni Fruit Juice* (North America and South West Pacific) (CXS 356R-2023). However, CCNASWP16 (2023) in forwarding this regional standard for adoption, noted the following outstanding issues:

   (i) **Issue 1**: The need to specify the type of solid-phase extraction cartridge, volume of water and methanol in a process of sample preparation for the thin-layer chromatography (TLC) method for the identification of scopoletin and deacetylasperulosidic acid.[2]

   (ii) **Issue 2**: The need to conduct verification studies for the high performance-liquid chromatography (HPLC) method to identify scopoletin and deacetylasperulosidic acid, noting that the HPLC method was still incomplete and investigation to validate it was underway.[3]

   (iii) **Issue 3**: The identified data gap for conducting a safety evaluation of scopoletin and need to consider how data needed for a safety evaluation of scopoletin could be provided.[4]

2. Regarding the first two outstanding issues, it was noted that the methods of analysis would be forwarded to CCMAS for endorsement once outstanding issues for each method of analysis were resolved by CCNASWP.[5]

3. CCNASWP16 agreed to task the Coordinator to work with the Members in the NASWP region to resolve the first two outstanding issues (specifically specification of the solid-phase extraction cartridge and the high-performance liquid chromatography (HPLC) method to identify scopoletin and deacetylasperulosidic acid) in order to forward the methods of analysis to CCMAS42 (2023) for endorsement.[6]

4. To address the third outstanding issue, CCNASWP16 agreed to request the Codex Committee on Contaminants in Foods (CCCF) to keep scopoletin in the priority list for evaluation by the Joint Expert Committee on Food Additives (JECFA) and to provide further data as it becomes available, and encouraged Members of the region to generate and submit data to GEMS/Food.[7]

## Current progress on outstanding issues

Issue 1: TLC methods for the identification of scopoletin and deacetylasperulosidic acid

5. The outstanding issues have been resolved and CCMAS42 endorsed the methods of analysis for scopoletin and for acetylasperulosidic acid based on TLC, for inclusion in the *Recommended Methods of Analysis and Sampling* (CXS 234-1999).[8]

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[1] This document was prepared by the Group of Experts of the Global Food Regulatory Science Society (GFoRSS), the Disciplinary Group for the International Union of Food Science and Technology (IUFoST).
[2] REP23/NASWP paragraph 69
[3] REP23/NASWP paragraph 66
[4] REP23/NASWP paragraph 71
[5] REP23/NASWP paragraph 65
[6] REP23/NASWP paragraph 73(ii)
[7] REP23/NASWP paragraphs 73(iii) and (iv)
[8] REP23/MAS paragraph 29

Issue 2: HPLC methods for the identification of scopoletin and deacetylasperulosidic acid

6. Noting that the main issue for data generation and safety assessment is scopoletin, an overview of efforts underway to develop and validate the HPLC method(s) for scopoletin in noni fruit juice, and reports on the performance of the HPLC method can be found in paragraphs 6-13 of the Annex.

Issue 3: Data for the safety evaluation of scopoletin by JECFA

7. An approach forward to support the generation of data on scopoletin in noni fruit juice using validated methods is proposed in paragraphs 17-21 of the Annex.

8. Paragraphs 17-21 of the Annex also offers perspectives for collaborative work to be initiated amongst Members in the NASWP region and Observers to generate data on the natural occurrence of scopoletin in noni fruit juice available for sale in South West Pacific markets.

9. The data generated could be submitted for consideration by JECFA to support the safety evaluation of scopoletin in noni fruit juice.

**Use of liquid-chromatography-mass spectrometry (LC-MS) methods for the identification of scopoletin**

10. In addition to the TLC and HPLC methods for the identification of scopoletin in noni fruit juice, the possible use of liquid chromatography-mass spectrometry (LC-MS) methods for the identification of scopoletin in noni fruit juice and related products is highlighted in paragraphs 14-16 of the Annex.

**Recommendation**

11. CCNASWP17 is invited to:

    a. note the progress made in addressing the outstanding issues related to the HPLC method for the identification of scopoletin in noni fruit juice;

    b. consider recommending method(s) of analysis for the identification of scopoletin in noni fruit juice based on the LC-MS principle for CCMAS' endorsement, to be included in the *Recommended Methods of Analysis and Sampling* (CXS 234-1999); and

    c. encourage Members in NASWP region to generate data on scopoletin in noni fruit juice using validated methods and submit this data to GEMS/Food to support a future safety evaluation of scopoletin by JECFA.

**Annex**

## DISCUSSION PAPER: UPDATE ON THE METHODS OF ANALYSIS OF SCOPOLETIN IN NONI FRUIT JUICE[9]

### A. Introduction to scopoletin in noni fruit juice

1. *Morinda citrifolia* L., commonly known as Noni, is a Rubiaceous plant widely distributed in many tropical and subtropical regions. Noni has been extensively used as food and folk medicine for the prevention or improvement of diversified health problems by Polynesians for more than 2000 years (Chan-Blanco et al., 2007). Hundreds of phytochemical components were isolated from Noni, including flavonoids, anthraquinones, iridoids, and glycosides. These phytochemicals receive continuous attention for their variety of medicinal value and health-care functions, such as antioxidant, antimicrobial, analgesic, anti-inflammatory, anticancer, antidiabetic, cardiovascular protection and immunoregulation (Basar & Westendorf, 2011; Tasfiyati et al., 2022; Wang et al., 2002).

2. Scopoletin or 6-methoxy-7-hydroxycoumarin (**Figure 1**), a key naturally occurring phytochemical in Noni, has been reported to exhibit antioxidative, antimicrobial, anti-inflammatory properties, and contribute to blood pressure regulation. Additionally, it has been proposed as a marker for the identification and quality control of Noni and its derivative products (Tasfiyati et al., 2022).

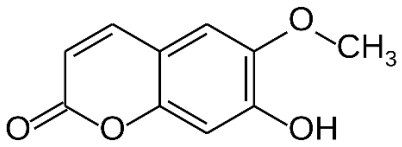


***Figure 1**: Chemical structure of scopoletin.*

3. Noni fruit juice is traditionally made by fermentation of mature Noni fruits in sealed containers for more than a month or longer at ambient temperature. This noni derived product is one of the main exports for some Pacific Island Countries (PICs).

4. Currently, no clinical studies have determined the potential toxicity level of scopoletin in humans. However, some reports have indicated possible toxicity. As a precaution, scopoletin levels should be maintained As Low As Technologically Feasible until the Joint FAO/WHO Expert Committee on Food Additives (JECFA) establishes a safe threshold. On an interim basis, a maximum level of **150 ppm** was identified as a sentinel level of scopoletin in noni fruit juice samples.

5. In this context, there is an urgent need for accurate and representative data on scopoletin levels in noni fruit juice to facilitate an informed assessment by JECFA and the establishment of a safe and technologically achievable Maximum Level. Implementing a fit-for-purpose analytical method is essential to support this critical data generation effort effectively.

### B. High Performance Liquid Chromatography (HPLC) Based Analytical Approaches for the Determination of Scopoletin in Noni Fruit Juice

*High Performance Liquid Chromatography - Photodiode Array Method (HPLC-PDA)*

6. HPLC-PDA method (photodiode array -PDA- detector, also known as diode array detector - DAD) was proposed as a method of choice for the qualitative and quantitative analysis of scopoletin in noni fruit juice, being a well-established method for the identification and quantification of coumarins[10]. The Reversed-phase (RP) mode is used in HPLC, employing a non-polar stationary phase (mostly C18) and achieving the separation between the targeted analyte, which is scopoletin in this case, and the remaining interfering matrix components after sample preparation and clean-up steps. The PDA detector is a detector that uses the principle that the target analyte encloses a chromophore, absorbing light in the UV or visible range, which is the case of scopoletin (**Figure 2**).

[9] This discussion paper is the result following the recommendation of the Second Codex Colloquium for North America and the Southwest Pacific hosted by Fiji from 26-28 February 2024.

[10] Coumarins are a highly promising group of bioactive heterocyclic compounds belonging to the family of benzopyrones (1,2-benzopyrones or 2H-1-benzopyran-2-ones). Scopoletin is a hydroxycoumarin with a molecular weight of 192.7 g/mol and an empirical formula of $C_{10}H_8O_4$ (Gao et al., 2024)

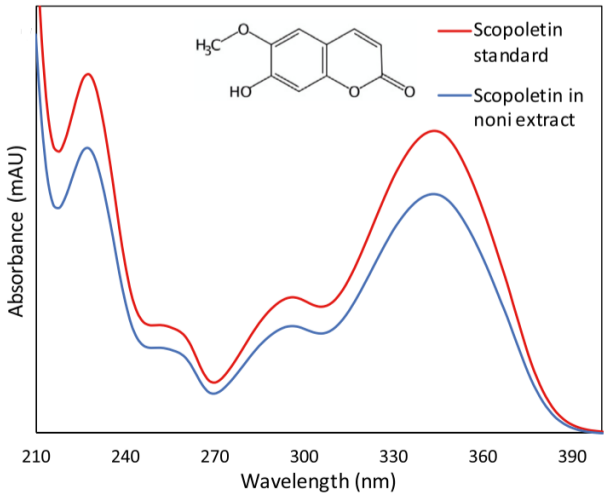


***Figure 2**: UV spectra of scopoletin standard and scopoletin spiked in noni extract (Tasfiyati et al., 2023).*

7. **Table 1** presents a selective literature review on the use of HPLC-PDA for analyzing scopoletin levels in noni derived products. As shown, all HPLC methods utilized operate in reverse-phase mode with a C18 column and employ common liquid chromatography mobile phases (Organic solvent combined to an acidified aqueous solution) in gradient mode. A range of Noni products was analyzed, with scopoletin concentrations varying widely, influenced by factors such as fruit ripeness, processing methods, and geographic origin. In most cases, scopoletin levels did not exceed 250 ppm.

8. Sample preparation steps ranged between solvent extractions for solid Noni products (powder, capsules, tea, etc.) to simple dilution and filtration in the case of liquid noni fruit juice. Basar & Westendorf, 2011 proposed however a solvent extraction step for the juice, which might introduce a further clean-up step before HPLC analysis.

***Table 1**: Literature review on the application of HPLC-PDA for the determination of scopoletin in noni derived products.*

| Samples | Sample preparation | HPLC-PAD parameters | Levels of scopoletin | Reference |
|---|---|---|---|---|
| Lyophilized ripped Noni fruits | • Ultrasound assisted extraction in 25 mL MeOH.<br>• Evaporation till dryness in rotary evaporator.<br>• Reconstitution in 2 mL MeOH | C18 column, Mobile phase (ACN/0.3% FA), gradient mode, DAD | Scopoletin content was found to be low in unripe fruits but increases during ripening (+/- 10 ppm) | (Chan-Blanco et al., 2007) |
| Noni fruit and commercial Noni fruit juice products | • **Noni fruit**: ultrasound assisted extraction (2 × 125 mL MeOH). Evaporation till dryness in rotary evaporator. Reconstitution in 10 mL MeOH.<br>• **Noni fruit juice**: 1 mL juice + 1 mL MeOH | C18 column, Mobile phase (ACN, MeOH, 0.1% TFA), gradient mode, DAD | • Noni fruit juice: 0.88–34.01 ppm | (Deng et al., 2010) |
| Commercial Noni fruit juice | Vortex assisted extraction with 0.5 mL EtOAc, centrifugation, evaporation till dryness and reconstitution with 100 µL EtOH | C18 column, Mobile phase (ACN, 0.1% TFA), gradient mode, 254 nm | 9-235 ppm | (Basar & Westendorf, 2011) |

| | | | | |
|---|---|---|---|---|
| Fermented and non-fermented *M. citrifolia L.* extracts | Freeze drying followed by sonication in distilled water | C18 column, Mobile phase (ACN, 0.1% FA), gradient mode, DAD | • Non-fermented: N.D<br>• Fermented: 1010 ppm | (Choi et al., 2021) |
| Noni powder and noni based products (capsule, powder, and tea | Accelerated solvent extraction (ASE) or maceration with EtOH, evaporation till dryness and reconstitution with 3 mL MeOH | C18 column, Mobile phase (ACN, 0.1% FA), gradient mode, DAD | • Noni powder: 29.23 ± 0.64 ppm<br>• Noni capsule: 219.73 ± 3.74 ppm<br>• Noni tea: 45.46 ± 0.47 ppm | (Tasfiyati et al., 2022) |
| Noni capsules, instant powder, teas, and effervescent | Accelerated solvent extraction (ASE) or maceration or ultrasound assisted extraction (UAE) or microwave assisted extraction (MAE) with EtOH, evaporation till dryness and reconstitution with 3 mL MeOH | C18 column, Mobile phase (ACN, 0.1% FA), gradient mode, DAD | • Capsules: N.D – 219.73 ppm<br>• Instant powder: N.D – 29.23 ppm<br>• Tea: 45.46 – 95.09 ppm<br>• Effervescent: N.D | (Tasfiyati et al., 2023) |

**: N.D: not detected or below limit of detection*
***: ACN (Acetonitrile); EtOAc (Ethyl Acetate); EtOH (Ethanol); FA (Formic Acid); MeOH (Methanol); TFA (Trifluoroacetic Acid)*

*Preliminary Development and Validation of a HPLC-PDA Method to Determine scopoletin in noni fruit juice*

9. Scientists from the Government of Samoa developed a methodology for the determination of scopoletin in noni fruit juice. This method was based on the HPLC-PDA reported by Choi et al., 2021. The method was validated following a single-laboratory validation procedure at the Scientific Research Organisation of Samoa (SROS), in terms of linearity, precision, accuracy, limit of detection (LOD) and of quantification (LOQ).

10. The linearity of the method was investigated in the ranges of 1 to 200 mg/L ($R^2$=0.998) and 1 to 1000 mg/L ($R^2$=0.994). The accuracy verification showed recoveries in spiked samples in the acceptable range of 90 – 110%. Regarding the precision in terms of repeatability and intermediate repeatability, all relative standard deviations (RSD) were below the acceptable limit of 10%. The LOD and LOQ estimated for the method were 0.04 and 0.4 mg/L, respectively.

11. Following the presentation of these results in a validation report by the SROS, Australia provided an assessment of the validated method and raised few issues that could be summarized in the following:

- The method's applicable range, which will depend on its intended purpose: either for compliance assessment with the sentinel level of 150 ppm or for general data generation on the occurrence of scopoletin in fermented noni fruit juice.
- The linearity range of the method, with Australia recommending adherence to a 1–200 mg/L range to improve model predictability. They also emphasized the importance of evaluating residual errors across this linear range.
- The recovery experiment to assess the method's accuracy, where Australia highlighted the need to consider higher spiking levels to align with the naturally occurring scopoletin levels in the reference samples.

12. Australia also proposed incorporating additional information into the validation report, specifically:

- sample preparation and chromatographic conditions; and
- the evaluation of specificity, ruggedness, and uncertainty through additional experiments, with a particular focus on the method's specificity against other natural and similar compounds that may be present in the juice and could interfere with the determination of scopoletin.

13. The developed HPLC-PAD method is well-established and suitable for the determination of scopoletin in fermented noni fruit juice. However, certain issues remain unresolved, particularly regarding the method's specificity, as interferences from other naturally occurring compounds, namely deacetylasperulosidic acid, asperulosidic acid, scopoline, asperuloside, etc. may affect the accurate quantification of scopoletin.

*Liquid Chromatography – Mass Spectrometry*

14. Liquid Chromatography-Mass Spectrometry (LC-MS) has become a powerful analytical tool due to its ability to combine the separation efficiency of liquid chromatography (LC) with the detection specificity and sensitivity of mass spectrometry (MS).

15. Single quadrupole MS systems provide straightforward mass detection and are highly effective for routine analysis where basic mass information is sufficient. In contrast, triple quadrupole systems enhance analytical capabilities by enabling multiple reaction monitoring (MRM), which significantly improves selectivity and sensitivity by targeting specific ion transitions.

16. This level of selectivity would be especially valuable for scopoletin analysis, as it allows for the accurate identification and quantification of scopoletin even in complex matrices, such as fermented noni fruit juice, overcoming potential interferences that may challenge HPLC-PDA methods. Unlike HPLC-PDA, which relies solely on UV-Vis absorbance and may struggle with co-eluting compounds, LC-MS provides robust molecular-level insight, making it a superior choice for quality control and detailed characterization of noni fruit juice and related products.

***Table 2**: Literature review on the application of LC-MS for the determination of scopoletin in noni derived products.*

| Samples | Sample preparation | LC-MS parameters | Levels of scopoletin | Reference |
|---|---|---|---|---|
| Noni Fruit Powder and Commercial Noni-Derived Products | • Solid samples: solvent extraction assisted by ultrasound or ASE with MeOH<br>• Juice samples: 1:10 aqueous dilution | ESI-, C18 column, Mobile phase 0.1% formic acid / CAN, MS scan mode (m/z 170 to 700) | Noni fruit juice: N.D - 23.2 ppm | (Potterat et al., 2007) |
| Noni fruit juice | • Extraction with Ethyl Acetate.<br>• Concentration using a rotary evaporator<br>• Reconstitution with MeOH : Water (1:1, v/v) | ESI (-) MS/MS, C18 column, Mobile phase ACN-MeOH -0.05% of Acetic acid<br>MRM mode (191.0338>176.0110) | 2.01-171.21 ppm | (Yan et al., 2018) |

### C. Plans Forward for the Generation of Data Related to the Occurrence of Scopoletin in Noni Fruit Juice Products

17. It is imperative to document the occurrence levels of scopoletin in noni fruit juice. To that end, the reliance upon validated quantitative methods will be required. Both the HPLC-PDA and LC-MS methods offer analytical protocols that can be applied for this purpose.

18. A request will be made for CCNASWP Members and their stakeholders to share existing data, including a detailed description and characterization of the analytical protocols employed. In addition, a collaborative initiative will be launched to support a renewed effort to collect data generated for scopoletin in noni products available in the region through the implementation of an LC-MS based method.

19. This effort will include a capacity building component, where:

- a newly developed analytical protocol will be shared for multi-laboratory verification, and dissemination amongst laboratory operators in the region; and
- an on-site training will be organized in collaboration with technology providers and the South-West Pacific Section of AOAC INTERNATIONAL.

20. Support will be sought for sample pick-up and shipment to one or more analytical testing sites, where the validated LC-MS method will be deployed. An expert team, gathering representatives of analytical testing institutions of the region will be called upon to evaluate the method validation data and the occurrence levels generated through this approach.

21. Once reviewed, the data will be readied for submission to the GEMS/Food Database, as a pre-requisite for the prioritization of the evaluation of scopoletin by JECFA.

**References**


1. Basar, S., & Westendorf, J. (2011). Identification of (2E,4Z,7Z)-Decatrienoic Acid in Noni Fruit and Its Use in Quality Screening of Commercial Noni Products. *Food Analytical Methods*, *4*(1), 57–65. https://doi.org/10.1007/s12161-010-9125-9

2. Chan-Blanco, Y., Vaillant, F., Pérez, A. M., Belleville, M. P., Zúñiga, C., & Brat, P. (2007). The ripening and aging of noni fruits (Morinda citrifolia L.): Microbiological flora and antioxidant compounds. *Journal of the Science of Food and Agriculture*, *87*(9), 1710–1716. https://doi.org/10.1002/jsfa.2894

3. Choi, S. Il, Kwon, H. Y., La, I. J., Jo, Y. H., Han, X., Men, X., Lee, S. J., Kim, Y. D., Seong, G. S., & Lee, O. H. (2021). Development and validation of an analytical method for deacetylasperulosidic acid, asperulosidic acid, scopolin, asperuloside and scopoletin in fermented morinda citrifolia l. (noni). *Separations*, *8*(6). https://doi.org/10.3390/separations8060080

4. Deng, S., West, B. J., & Jensen, C. J. (2010). A quantitative comparison of phytochemical components in global noni fruits and their commercial products. *Food Chemistry*, *122*(1), 267–270. https://doi.org/10.1016/j.foodchem.2010.01.031

5. Gao, X.-Y., Li, X.-Y., Zhang, C.-Y., & Bai, C.-Y. (2024). Scopoletin: a review of its pharmacology, pharmacokinetics, and toxicity. *Frontiers in Pharmacology*, *15*. https://doi.org/10.3389/fphar.2024.1268464

6. Potterat, O., Von Felten, R., Dalsgaard, P. W., & Hamburger, M. (2007). Identification of TLC markers and quantification by HPLC-MS of various constituents in noni fruit powder and commercial noni-derived products. *Journal of Agricultural and Food Chemistry*, *55*(18), 7489–7494. https://doi.org/10.1021/jf071359a

7. Tasfiyati, A. N., Antika, L. D., Dewi, R. T., Septama, A. W., Sabarudin, A., & Ernawati, T. (2022). An experimental design approach for the optimization of scopoletin extraction from Morinda citrifolia L. using accelerated solvent extraction. *Talanta*, *238*. https://doi.org/10.1016/j.talanta.2021.123010

8. Tasfiyati, A. N., Antika, L. D., Septama, A. W., Hikmat, H., Kurniawan, H. H., & Ariani, N. (2023). A validated HPLC-DAD method and comparison of different extraction techniques for analysis of scopoletin in noni-based products. *Kuwait Journal of Science*, *50*(3), 276–281. https://doi.org/10.1016/j.kjs.2023.02.023

9. Wang, M.-Y., J West, B., Jensen, J., Nowicki, D., Su, C., K Palu, A., & Anderson, G. (2002). Morinda citrifolia (Noni): A literature review and recent advances in Noni research. *Acta Pharmacologica Sinica*, *23*(12).

10. Yan, Y., Lu, Y., Jiang, S., Jiang, Y., Tong, Y., Zuo, L., Yang, J., Gong, F., Zhang, L., & Wang, P. (2018). Quantitative determination of bioactive constituents in Noni Juice by High-performance liquid chromatography with electrospray ionization triple quadrupole mass spectrometry. *Pharmacognosy Magazine*, *14*(53), 70–75. https://doi.org/10.4103/pm.pm_74_17