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Simultaneous determination of acrylamide, Glycidamide, and Methylacrylamide in plant-based beverages using µ-dispersive solid phase extraction and HPLC-PDA: Development, validation, and occurrence

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ABSTRACT

Plant-based beverages are widely consumed as dairy alternatives due to their nutritional value and sustainability. However, thermal processing may lead to the formation of acrylamide and related toxicants. This study developed and validated a miniaturized dispersive solid-phase extraction (µ-dSPE) method coupled with high-performance liquid chromatography and ultraviolet detection for the simultaneous determination of acrylamide, glycidamide, and methacrylamide, using thiourea as an internal standard. Unlike existing µ-dSPE/QuEChERS and MS-based approaches, the workflow is entirely aqueous (no organic solvents) and requires no derivatization, aligning with green analytical chemistry. Optimization was conducted using a $3^4 4^1$ screening design and response surface methodology. The method showed excellent linearity ($r^2 > 0.999$), low detection limits (0.24–0.98 µg/mL), and excellent accuracy (91–111 % recovery) and precision (RSD < 6.1 %). Analysis of 15 commercial beverages revealed acrylamide in 14 samples (0.33–1.17 µg/mL), especially in oat and mixed-protein types; glycidamide and methacrylamide were not detected. Environmental assessment using AGREEprep yielded a high greenness score (0.72) and a high practical applicability score (80.0) by the Blue Applicability Grade Index. This µ-dSPE-HPLC-PDA method provides a reliable, cost-effective, and environmentally-friendly approach for monitoring heat-induced contaminants in plant-based beverages.

1. Introduction

Plant-based beverages (PBBs) have emerged as prominent alternatives to dairy products, driven by increasing consumer demand for sustainable and health-promoting food options (McClements et al., 2019). Although dairy products provide a complete nutritional profile, PBBs are distinguished by their rich content of phytochemicals with proven bioactivity, including compounds with antioxidant, anti-inflammatory, and potentially beneficial effects on cardiovascular and metabolic health (Xiao et al., 2022). Additionally, they offer viable options for individuals with lactose intolerance, low-cholesterol diets, or milk protein allergies (de Oliveira Ribeiro et al., 2020; Szparaga et al., 2019). Recent data from the Spanish Ministry of Agriculture, Fisheries and Food (Ministerio de Agricultura, Pesca y Alimentación, 2024) indicate a steady increase in the consumption of PBBs in Spain from 2017 to 2023. According to the annual food consumption reports, total PBB consumption rose from approximately 226 million liters in 2017 to over 289 million liters in 2023. Notably, the consumption of organic PBBs, which began to be recorded separately in 2020, reached over 31 million liters in its first year and maintained significant levels in subsequent years. This upward trend underscores the growing relevance of PBBs as functional food options in the Spanish market (MAPA, 2024).

Typically formulated using a diverse range of raw materials, including legumes, nuts, cereals, and seeds, PBBs undergo multiple

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processing steps aimed at replicating the sensory and nutritional attributes of conventional milk products. These processes generally include soaking, grinding, and heat treatments such as pasteurization or Ultra-High Temperature (UHT) (da Silva Matos et al., 2024). Roasting of raw materials, in particular, is a critical step for flavor enhancement but also promotes Maillard reactions that may lead to the formation of acrylamide (AA) and glycidamide (GA), whereas methacrylamide (MA) formation is more commonly associated with interactions between packaging materials and food components during UHT processing (Cheng et al., 2018; Custodio-Mendoza et al., 2024;Fan et al., 2023; Pucci et al., 2024).

AA is predominantly formed via the Maillard reaction, involving reducing sugars and free asparagine under high temperatures (>120 °C), a process extensively studied in cereal-based products, coffee, and fried foods but less so in aqueous matrices like PBBs (Fan et al., 2023; Pucci et al., 2024). The International Agency for Research on Cancer (IARC) classifies AA as Group 2 A, meaning it is "probably carcinogenic to humans" (World Health Organization, 2023). The European Food Safety Authority (EFSA) has set a benchmark dose limit (BMDL10) for AA at 0.17 μg/kg bw/day for risk assessment purposes, though this is not a strictly tolerable daily intake (Hemgesberg et al., 2021). Given the high moisture content of PBBs, the detection of AA is unexpected. However, Pucci et al. (2024) reported its presence, suggesting raw materials as potential sources. GA, a metabolite of acrylamide, is also classified as Group 2 A by IARC due to its potential carcinogenicity (World Health Organization, 2023). No established TDI has been set for GA or MA yet, emphasizing the need for reliable analytical methods to monitor its occurrence in food matrices. Despite GA being a known genotoxic metabolite of AA, and MA being associated with food packaging, their occurrence in PBBs has not been assessed yet, highlighting a critical research gap.

The analysis of AA, GA, and MA in food matrices typically involves sample preparation techniques such as QuEChERS, dispersive solid-phase extraction (dSPE), and micro-dispersive solid-phase extraction (μ-dSPE), coupled with chromatographic techniques like high-performance liquid chromatography (HPLC) or gas chromatography (GC) with various detection systems (Custodio-Mendoza et al., 2024; Della Posta et al., 2025; Sebastia et al., 2023). In a recent study, Pucci et al. (2024) determined AA in PBB by extracting it from water-based extracts of freeze-dried samples, followed by cleanup using Carrez reagents and solid-phase extraction, with final detection via LC-MS/MS. However, these traditional approaches often require extensive derivatization, increasing solvent use and processing time, which contradicts the principles of green analytical chemistry (López-Lorente et al., 2022; Sajid & Płotka-Wasylka, 2022).

Recent trends in green analytical chemistry emphasize the development of miniaturized sample preparation methods that reduce solvent consumption, enhance extraction efficiency, and align with sustainable laboratory practices. For instance, Della Posta et al. (2025) employed a miniaturized matrix solid-phase dispersion (MSPD) for AA determination in bread using deep eutectic solvents (DES), while Custodio-Mendoza et al. (2024) reported a modified QuEChERS-DLLME method for simultaneous AA and GA determination in infant formula. Nevertheless, existing methods primarily target solid matrices, overlooking the complexities of aqueous systems like PBBs, where analytes may exist at trace levels and exhibit matrix effects that complicate detection and quantification (Custodio-Mendoza et al., 2024; Della Posta et al., 2025). In dispersive micro-solid phase extraction (μ-dSPE) offers an efficient, rapid, and green alternative, as it enhances mass transfer and sorption kinetics while reducing solvent and sorbent consumption—advantages particularly well-suited for trace-level analyte extraction in complex liquid matrices (Chisvert et al., 2019).

Recent advances in green, miniaturized sample preparation for aqueous and semi-liquid foods, including milk/infant formula, fruit juices, herbal teas, yogurts, honey, wines and beers, have showcased sorbent- and solvent-lean strategies such as SPME/μ-SPE, DES- or NADES-based microextractions, and composite sorbents (e.g., DES@-MOF) tailored for liquid matrices (De Cesaris et al., 2024; Martínez-Pérez-Cejuela & Gionfriddo, 2024). These workflows report low solvent volumes, reusable phases, and direct coupling to HPLC/GC systems; for instance, DES@MOF sorbents have enabled efficient extractions in herbal teas, fruit/vegetable liquids, and yogurts with sub-mL elution (Deng et al., 2024). Complementary reviews on NADES emphasize replacing organic solvents in food-contaminant extraction while keeping methods simple and safer for routine labs (Luque-Uría et al., 2025). Finally, SPME remains a leading solvent-free option for water and food liquids (e.g., juices, honey), aligning greenness with preconcentration performance (Poojary et al., 2025).

Thus, this study aims to address these gaps by developing and validating a novel μ-dSPE method coupled to HPLC-PDA (photodiode array detection) for the simultaneous determination of AA, GA, and MA in PBBs, a matrix and analyte combination not previously explored in the literature. The proposed method departs from conventional μ-dSPE and QuEChERS-type extractions by operating entirely in an aqueous medium, without organic solvents or derivatization, thereby enhancing its environmental and operational simplicity. It also introduces thiourea (Th) as an internal standard, offering a practical alternative to isotopically labelled compounds, which are typically required in MS-based analyses but incompatible with UV detection. By integrating miniaturization, solvent-free extraction, and direct UV detection in adherence to green analytical chemistry principles, this approach provides a sustainable, cost-effective, and analytically robust strategy for monitoring heat-induced contaminants in PBBs.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals used were high purity (>99 %), unless stated otherwise. Acetic acid (CAS 64–19-7) and ammonium acetate (CAS 631–61-8) were purchased from Merck (Darmstadt, Germany). Sodium chloride (CAS 7647-14-5) and formic acid (CAS 64–18-6) were obtained from Poch (Gliwice, Poland). O-Phosphoric acid (85 %, CAS 7664-38-2) and anhydrous citric acid (CAS 77–92-9) were supplied by Chempure (Piekary Śląskie, Poland). Ammonium formate (CAS 540–69-2) was purchased from Hadron Scientific (Kielce, Poland), and ammonium sulfate (CAS 7783-20-2) from Carl Roth (Karlsruhe, Germany). Various SPE bulk sorbents, including Bond Elut EMR-Lipid (EMR-Lipid), C18, primary secondary amine (PSA), Florisil, and Silica Strong Anion Exchange (Si-SAX), were supplied by Agilent® (Santa Clara, CA, USA). OASIS MCX and OASIS HLB were purchased from Waters® (Milford, MA, USA). Mesoporous silica SBA-15 and Supel™ QuE *Z*-Sep + bulk (Z-Sep+) were obtained also from Merck. Phenomenex (Torrance, CA, USA) supplied Strata CN, Strata NH2, and Strata X sorbents. Sorbents not available in bulk were procured as SPE columns, and the sorbent material was carefully recovered from these columns in the laboratory.

Analytical standards of AA (CAS No. 79–06-1), GA (CAS No. 7659-86-1), MA (CAS No. 79–39-0), and Th (≥99.0 %, CAS No. 62–56-6) were also obtained from Merck. Stock solutions were prepared by dissolving each compound in ultrapure water at defined concentrations. All solutions were stored at 4 °C and used within one week to ensure stability. This study used different laboratory instruments, including an analytical balance (Mettler Toledo, model ME104, Spain), a centrifuge (Hettich, model 320/320 R), a vortex mixer (ChemLand, model MX-S), and a pH meter (Crison Instruments, model Basic 20, Barcelona, Spain), to perform all experiments.

2.2. Samples

Fifteen plant-based, non-dairy milk alternatives were purchased from local stores in the Warsaw metropolitan area (Poland). The selection included coconut, oat, rice, almond (roasted and non-roasted), and mixed varieties (coconut-soy and oat-hazelnut). All samples were ultra-high-temperature (UHT) treated and free from added sugars, flavorings, and gluten.

The specific compositions of the samples were as follows: four coconut-based milks—one containing 5.7 % coconut protein and 2.9 % soybean protein, another with 3.5 % coconut and 12 % oat, one with 8.6 % coconut, and another with 8.5 % coconut; five oat-based milks—containing 15 %, 13 %, 8.7 %, 10 %, and 10.3 % oat, respectively; two rice-based milks—one with 10 % rice and the other with 16 % rice; three almond-based milks—one made from non-roasted almonds (5 %) and two from roasted almonds (2.3 and 4 %); and one mixed milk with 15 % oat and 1.2 % hazelnut.

All samples were stored in their original packaging at room temperature, protected from light and moisture until analysis. After opening, they were transferred into 15 mL vials with septa, purged with N_2 to eliminate oxygen and prevent oxidation, and stored at 4 °C in a refrigerator, protected from light for not longer than one week.

Quality control (QC) samples were prepared using a non-roasted almond beverage as the blank matrix. The samples were spiked with known concentrations of the target analytes at three levels: 1 μg/mL (low QC), 2 μg/mL (medium QC), and 5 μg/mL (high QC).

2.3. *μ-Dispersive solid phase extraction*

A diagram of the μ-dSPE procedure is shown in Fig. 1. In summary, 1 mL of a PBB was transferred to a 5 mL conical-bottom plastic Falcon tube. To this, 0.2 g of ammonium sulfate, 20 μL of a 2 % acetic acid solution (to achieve a final acetic acid concentration of 6 mM in the sample), and 0.2 mL of 1 μg/mL Th as an internal standard (IS) were added. The mixture was vortexed for 2 min at 1500 rpm, followed by centrifugation for 5 min at 9000 rpm to separate the proteins.

After centrifugation, 500 μL of the aqueous phase was transferred to a second 5 mL conical-bottom plastic Falcon tube containing 40 mg of PSA. This system was vortexed for 6 min at 1500 rpm to perform the clean-up step and then centrifuged at 9000 rpm for 5 min. The resulting aqueous extract was filtered through a 0.22 μm PTFE syringe filter and transferred to a vial with an insert for HPLC-PDA analysis. The total extraction process required 20 min.

2.4. *High-performance liquid chromatography – PDA determination*

Chromatographic separation was carried out using a ThermoFisher Scientific (Waltham, MA, USA) system consisting of an Accela Autosampler (60057–60,020), a quaternary pump (600), and a photodiode array (PDA) detector operating at 40 Hz with a 5 cm LightPipe™ flow cell. The separation was based on the method described by Ikhsan et al. (2022), with key modifications including the inclusion of MA as a target analyte and the use of Th as an internal standard—both not previously reported. Separation was achieved on a 3 μm Luna Omega C18 column (4.6 × 100 mm) from Phenomenex (CA, USA). An isocratic mobile phase of 0.1 % O-phosphoric acid aqueous solution and methanol (97:3 *v*/v) with a 0.5 mL/min flow was used. The injection volume was set to 10 μL, and the total analysis time was 20 min. The PDA detector simultaneously scanned from 190 to 600 nm to capture the full UV spectrum of the analytes while specifically monitoring wavelengths at 190, 193, 197, and 232 nm.

2.5. *Experimental design and statistical analysis*

All statistical analyses were performed using R software (version 4.5.0, R Core Team, Vienna, Austria). A descriptive analysis was conducted to summarize analytes content across all samples, reporting mean, median, range, and standard deviation values. Boxplots were generated to visualize the distribution of acrylamide content across different protein types (coconut, oat, rice, almond). Subsequently, a Kruskal-Wallis test was performed to assess potential differences in acrylamide levels among the different sample groups. Post hoc comparisons were conducted using Dunn's test with Bonferroni adjustment. Finally, a correlation analysis was conducted to assess the potential relationship between protein content (%) and analyte content (μg/mL) using Pearson and Spearman correlation coefficients.

Factor-by-factor optimization was initially applied to establish a starting point for further refinement of the μ-dSPE procedure. The experimental design, evaluation, and graphical analysis were carried out using NemrodW® statistical software (Mathieu et al., 2000). Based on the preliminary results, an asymmetrical screening design ($3^4 4^1$) was employed to evaluate the effects of five variables at different levels. Three variables (ammonium salt as a salting-out agent, clean-up sorbent amount and type, and clean-up time) were studied at three levels, while the type of acid for protein precipitation was studied at four levels. This design was tested through 12 experiments to assess its impact on the efficiency of the μ-dSPE procedure.

A Doehlert design was subsequently used to further investigate three significant factors in greater detail: clean-up time, sorbent amount, and sorbent composition. The Doehlert matrix included twelve experimental points plus three central points. Multi-criteria decision analysis with desirability functions was then applied to determine the optimal conditions by balancing multiple responses.

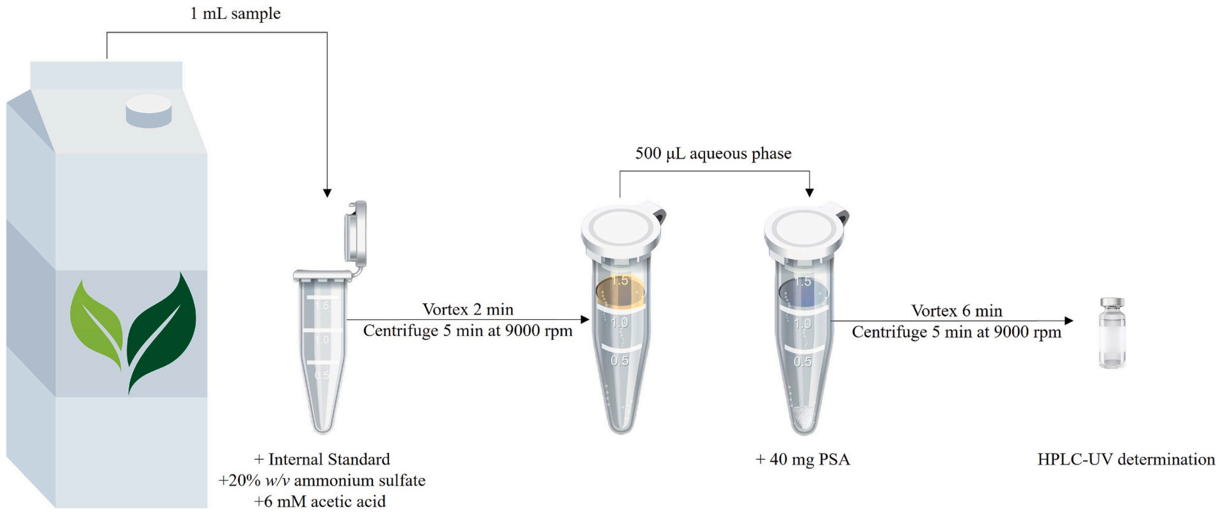

Fig. 1. Scheme of μ-Dispersive Solid Phase Extraction Coupled with HPLC-UV for Determination of Glycidamide, Acrylamide, and Methacrylamide in Plant-Based Beverages. PSA, Primary Secondary Amine clean up sorbent.

The sustainability of the sample preparation method was assessed using the AGREEprep tool, an open-access software designed for evaluating the environmental impact of analytical methods (Pena-Pereira et al., 2022). The AGREEprep evaluation is based on ten criteria, each scored between 0 (lowest performance) and 1 (highest performance). These scores, with default weightings for each criterion, are combined into an overall score ranging from 0 to 1, where 1 represents the highest level of environmental performance (Wojnowski et al., 2022). This tool identified areas for improvement, helping make the sample preparation method more eco-friendly. The practical applicability of the analytical method was assessed using the Blue Applicability Grade Index (BAGI), a freely available tool (https://bagi-index.anvil.app/) developed to evaluate the operational feasibility of analytical procedures within the framework of White Analytical Chemistry (Manousi et al., 2023). BAGI considers ten criteria related to analytical performance, instrumentation, throughput, automation, and material requirements. Each criterion is scored on a scale from 0 (lowest applicability) to 1 (highest applicability), and the individual scores are integrated into an overall index that reflects the method's practical potential for routine use.

2.6. *Analytical figures of merit*

The analytical figures of merit were evaluated using analytical standards of GA, AA, and MA, as well as Th as IS. Method validation followed FDA guidelines for chemical method validation in food, feed, cosmetics, and veterinary products (Food & Administration, 2018; U.S. Food and Drug Administration, 2019). The key parameters assessed were specificity, limits of detection (LOD), limits of quantification (LOQ), linearity, accuracy, and precision.

Method specificity was evaluated by ensuring a baseline separation of the target analytes in the enriched samples, using their maximum UV wavelength. This confirmed the method's ability to differentiate and quantify each analyte without interference from other substances in the matrix. Instrumental LOD and LOQ were determined using the Xcalibur™ software, with LOD and LOQ established at concentrations corresponding to 3 and 10 times the signal-to-noise (S/N) ratio, respectively.

Sample limits (LOD and lower limit of quantification, LLOQ) were calculated as 3.3 and 10 times the standard deviation of the blank response plus the mean blank response, respectively. These were determined using standard addition calibration curves with IS with at least six concentration levels for each analyte. The linear dynamic range extended from the LLOQ to the upper limit of quantification (ULOQ, 10 μg/mL).

QC samples at three concentration levels (1, 2, and 5 μg/mL) were used to evaluate the method's accuracy and precision. Accuracy was determined by calculating the recovery of each analyte from quintuplicate measurements ($n = 5$) at each level. Precision was assessed through both intraday and interday analyses, also using five replicates per level, with results expressed as percent relative standard deviation (%RSD). The obtained %RSD values confirmed the method's consistency and repeatability over time. The matrix effect was analyzed by comparing the slopes of external standard calibration curves with those of standard addition calibration curves. Method robustness was additionally verified by applying control chart analysis to the results obtained from the QC samples at the three concentration levels. The mean response and standard deviation of each level were used to construct Shewhart-type control charts, with warning limits set at mean ± 2 SD and decision (control) limits at mean ± 3 SD. This approach enabled the monitoring of variability and detection of potential trends or deviations from statistical control (warning and decision limits), confirming the method's stability and robustness under routine analytical conditions.

3. Results and discussion

3.1. *Optimization of sample preparation parameters for μ-dSPE*

In the economy of sample preparation procedures, sample size plays a crucial role, impacting not only the overall performance of the methodology but also its economic and environmental footprint (López-Lorente et al., 2022). Thus, finding a sample size that minimizes the method's impact without compromising its sensitivity is essential. In this work, three sample volumes were evaluated (Fig. 2.A), and it was found that for AA, all explored volumes (0.75, 1, and 1.5 mL) yielded similar chromatographic signals. However, for the other analytes and the internal standard (IS), the signal decreased when using 0.75 mL, with the effect being more pronounced for GA. This can likely be attributed to the inherently lower signal intensity associated with UV detection of GA without derivatization, making its quantification more susceptible to sample volume variations (Ikhsan et al., 2022). Therefore, 1 mL was selected for subsequent experiments.

Effective removal of matrix components that may interfere with analyte detection is crucial. To address this, the pre-cleaning stage focused on the elimination of proteins and fats from the PBBs by evaluating three different strategies: (i) protein precipitation through pH reduction below the isoelectric point using o-phosphoric acid, (ii) salting-out using ammonium sulfate, and (iii) a combined approach applying both acid-induced precipitation and salting-out. These strategies were compared to determine their effectiveness in improving analyte recovery and signal clarity. As shown in Fig. 2.B, for MA and the IS, decreasing the pH below the isoelectric point (using *o*-phosphoric acid) enabled an acceptable chromatographic response. However, this approach was not effective for GA and AA. This discrepancy can be explained by the fact that reducing the pH to the isoelectric point mainly induces protein precipitation but does not significantly affect fat removal, which may continue to interfere with the analysis (Gehring et al., 2011).

When only the salting-out effect (20 % *w/v* ammonium sulfate) was applied, acceptable signals were obtained for AA, GA, and the IS, although MA showed a lower chromatographic area compared to the isoelectric point strategy. Consequently, the combination of both approaches—acid-induced protein precipitation followed by salting-out—was assessed, achieving improved signals for all analytes. This enhancement is likely related to the synergistic effect of protein precipitation and the additional separation of the protein and insoluble fractions from the aqueous phase via the salting-out mechanism (Gehring et al., 2011).

The concentration of ammonium sulfate was then evaluated at 5, 10, 15, and 20 % *w/v* (Fig. 2.C). Although the IS showed similar signals across all concentrations, the rest of the analytes exhibited a marked decrease in signal as the salt concentration decreased, particularly GA, which dropped by more than 80 % at the lowest salt levels. Based on these results, 20 % *w/v* ammonium sulfate was selected for further experiments.

The selection of an appropriate clean-up agent is another critical step, especially in methodologies such as QuEChERS and SPE, where effective isolation of analytes is fundamental (Perestrelo et al., 2019). In traditional SPE applications for AA determination, clean-up is typically performed using Isolute Multimode, Oasis HLB, Bond Elut AccuCAT, and ChemElut cartridges (Sebastia et al., 2023). However, dSPE operates differently from conventional SPE, as it involves direct dispersion of the sorbent into the sample followed by centrifugation, rather than the percolation of the sample through a packed column (Salamat et al., 2025).

Given this operational difference, this study explored various silica-based sorbents ($n = 3$), including C18, EMR-Lipid, Florisil, Oasis HLB, Oasis MCX, PSA, mesoporous silica, silica SAX, Strata CN, Strata NH2, Strata X, and *Z*-sep+. As shown in Fig. 3, for the IS (Th), most sorbents achieved normalized chromatographic areas above 60 %. However, for

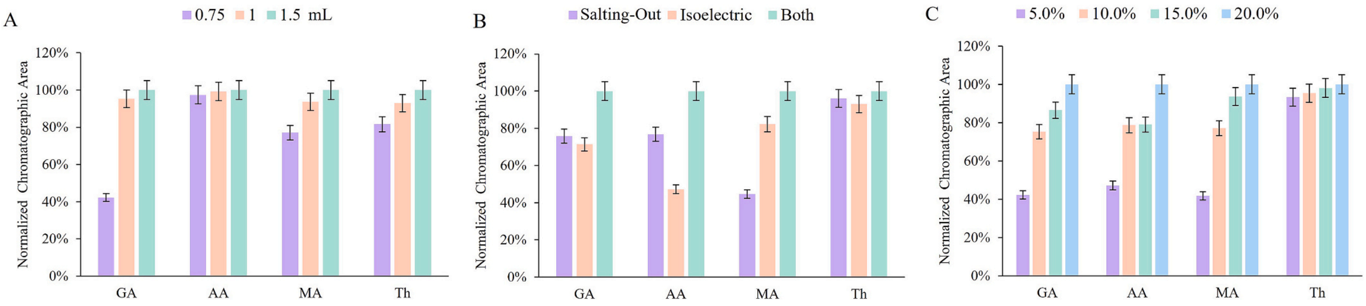

Fig. 2. Factor-to-Factor Optimization of Sample Size (A), Protein Precipitation Method (B), and Salt Concentration (C). GA, Glycidamide; AA, Acrylamide; MA, Methylacrylamide; Th, Thiourea (IS).

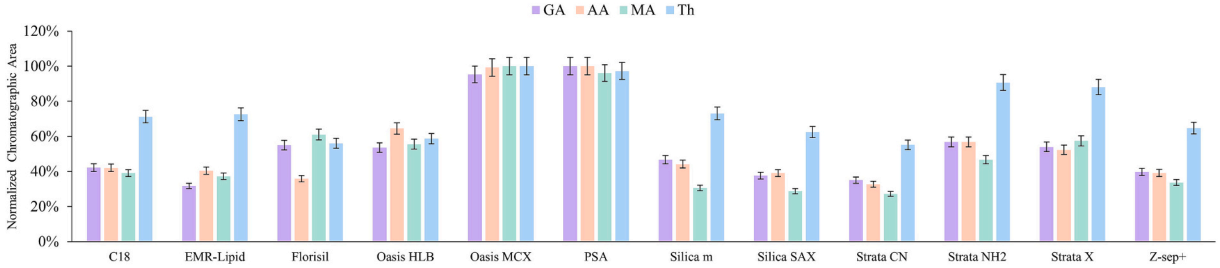

Fig. 3. Optimization of Sorbent Selection for Sample Clean-Up. GA, Glycidamide; AA, Acrylamide; MA, Methacrylamide; Th, Thiourea; m, mesoporous.

AA, GA, and MA, the majority of tested sorbents yielded chromatographic areas between 30 and 70 %. Error bars in the figure represent standard deviations (SD) from triplicate experiments, reflecting the reproducibility of the measurements; most values indicate low variability across replicates. The highest chromatographic signals for most analytes were achieved using Oasis MCX and PSA sorbents. This can be attributed to their sorbent properties: PSA, being a weak anion exchanger with primary and secondary amine groups, effectively removes polar interferences, while Oasis MCX, a mixed-mode cation exchanger, offers both reversed-phase and strong cation-exchange retention mechanisms (Andrade-Eiroa et al., 2016; Moral et al., 2023). These sorbents thus enhance the removal of matrix components without retaining the neutral amides of interest. Nevertheless, which sorbent was superior for this specific procedure was not entirely clear. Therefore, both PSA and Oasis MCX were selected for further optimization alongside other potentially influential parameters, using a multivariate optimization approach.

3.1.1. Multivariate optimization using experimental design

To identify the levels that favored the method's performance, an asymmetrical screening design ($3^4 4^1$) was employed, evaluating the effects of five variables: ammonium salt type as a salting-out agent (selected due to its effectiveness in protein and fat removal with reduced environmental impact and risk of precipitation in detection systems (Duong-Ly & Gabelli, 2014; Perestrelo et al., 2019; Olives, González-Ruiz and Martín, 2017), clean-up sorbent amount and type (PSA and Oasis MCX), clean-up time, and the type of acid for protein precipitation (exploring alternatives to halogenated acids).

This design was tested across 12 experiments to assess the impact of each factor on the efficiency of the μ-dSPE procedure. The results were analyzed using total effect (Fig. 4) and delta weight plots (Fig. S1). According to these graphs, the use of ammonium sulfate significantly increased the peak area for all analytes. This effect was statistically significant for GA and AA at the 95 % confidence level.

No statistically significant differences were found for the sorbent amount. Both 25 mg and 100 mg produced similar responses for most analytes.

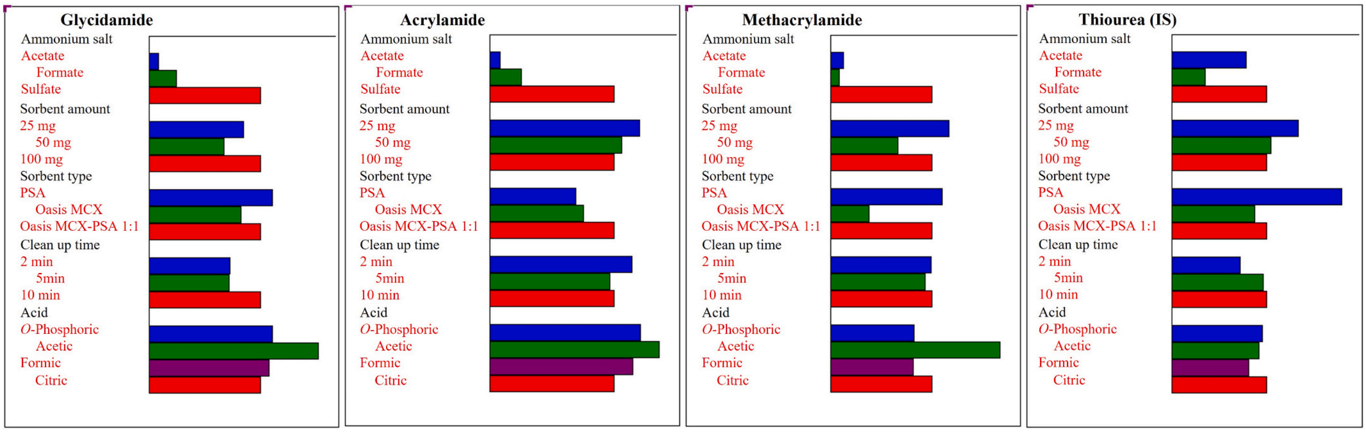

Fig. 4. Pareto Chart of the asymmetrical $3^4 4^1$ // 16 screening design.

Regarding sorbent type, the use of PSA improved the IS peak area (statistically significant effect with 95 % confidence level compared to the other levels). For the other analytes, no significant differences were observed, although slightly higher signals were achieved with PSA or the combination of Oasis MCX and PSA. For clean-up time, no statistically significant effect was found for any analyte. Similar signals were obtained across the tested levels for MA, while slight increases were observed for AA with 2 min and for the IS and GA with 10 min of clean-up time. No statistically significant differences were detected for the type of acid. However, a positive trend toward higher chromatographic signals was observed when using acetic acid, as shown in Fig. 4.

Based on the results of the screening design, ammonium sulfate was selected as the salting-out agent and acetic acid was chosen for protein precipitation, establishing these parameters for subsequent optimization. However, the optimal levels for parameters such as clean-up sorbent composition, amount, and clean-up time were not fully defined, necessitating further exploration using a response surface methodology. This approach allows the evaluation of a broader range of experimental points within the defined levels and the assessment of interaction effects between variables (which is particularly important given that sorbent amount and composition can influence the clean-up time required for effective matrix removal (Benedetti et al., 2019)). A Doehlert design was employed for this purpose.

The analysis of the surface response plots (Fig. 5) revealed a non-linear behavior of the system, with strong interactions between sorbent amount, PSA percentage, and clean-up time. In Fig. 5, the top row represents the clean-up time × sorbent amount surfaces, while the bottom row corresponds to PSA% × sorbent amount, with response (chromatographic peak area) coded by the color scale. The pronounced curvature and tilted “valleys” and “ridges” indicate true factor interactions rather than simple main effects. From a chemical perspective, lower sorbent amounts, and shorter times limit non-specific retention of the highly polar amides (AA, GA, MA), improving recovery, whereas increasing sorbent amount aids when matrix load is high but beyond 40 mg favors partial co-retention, particularly for GA due to its strong hydrogen-bonding ability. Specifically, lower sorbent amounts, and shorter clean-up times resulted in higher signals for AA, MA, and Th. A combination of low clean-up time and high sorbent amount enhanced signals for AA and GA, while low PSA percentage and high sorbent amount favored signal intensity for both AA and GA. Conversely, higher PSA percentage, which enhances removal of polar interferences via anion-exchange, combined with lower sorbent amount yielded improved signals for MA and Th. These results highlight the importance of balancing sorbent composition, quantity, and contact time to optimize analyte recovery. Based on these observations with different behaviors, the optimal conditions must be predicted using the multi-criteria decision analysis, based on desirability functions. These conditions were identified as 40 mg of sorbent composed entirely of PSA and a clean-up time of 6 min. This combination maximized the desirability across all analytes studied ensuring high recovery rates while maintaining process efficiency. The exclusive use of PSA at this amount allows for efficient removal of matrix interferences due to its strong affinity for polar impurities, while the selected clean-up time ensures sufficient contact without causing degradation or loss of analytes. These optimized parameters thus contribute to a sensitive, reproducible, and robust miniaturized dSPE method for the chromatographic determination of these amides.

3.2. *Analytical validation of the optimized μ-dSPE*

The selectivity of the method was supported by the UV spectra of the analytes recorded using analytical standards (Fig. S2). Each compound presented a distinct maximum absorbance wavelength and eluted at a different retention time (Table 1, Fig. S3), confirming their individual detection without signal overlap (Fig. S4). Specificity was further demonstrated by the analysis of a spiked non-roasted almond beverage sample, which showed no detectable interferences at the retention times corresponding to the analytes of interest (Fig. 6).

Sample limits were found to be adequate for the intended application, with LLOQs of 0.98 μg/mL, 0.24 μg/mL, and 0.69 μg/mL for GA, AA, and MA, respectively (Table 1). The method exhibited excellent linearity for all analytes over the tested concentration ranges (LLOQ–ULOQ), with determination coefficients (r^2) above 0.999.

The method showed satisfactory precision, with intraday and interday relative standard deviations (RSDs) below 6.1 % for all analytes, analyzed in quintuplicate at low (L-QC), medium (M-QC), and high (H-QC) quality control levels. For GA, intraday RSDs ranged from 3.0 % to 4.5 % and interday RSDs from 1.7 % to 4.2 %. For AA, intraday RSDs were between 2.5 % and 5.8 %, and interday RSDs between 0.9 % and 5.0 %. For MA, intraday RSDs varied from 3.8 % to 6.1 %, and interday RSDs from 1.9 % to 2.8 % (Table 1).

Accuracy, expressed as percentage recovery, was also analyzed in quintuplicate across the evaluated concentration levels of QC samples. Recoveries for GA were 91.4 %, 100.3 %, and 95.9 % at L-QC, M-QC, and

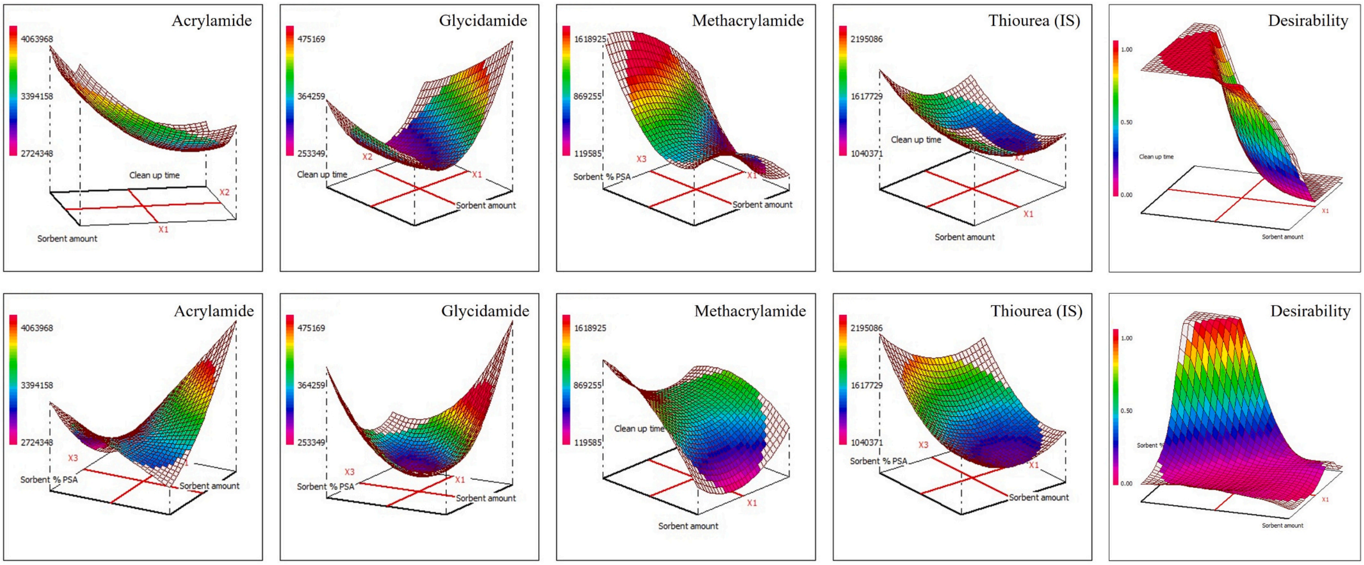

Fig. 5. Surface response from Doehlert design for clean-up time vs. sorbent amount (above) and % PSA sorbent vs. sorbent amount (below).

Table 1
Analytical Features of μ-Dispersive Solid Phase Extraction for Glycidamide, Acrylamide, and Methacrylamide HPLC-PDA Determination in Plant-Based Beverages.

Analyte	RT	λ_{UV} max	LOD	LLOQ	r^2	Intraday precision (n = 5) % RSD			Interday precision (n = 5) % RSD			Accuracy (n = 5) % Recovery		
	min		μg/mL	μg/mL		L-QC	M-QC	H-QC	L-QC	M-QC	H-QC	L-QC	M-QC	H-QC
Glycidamide	3.07	190	0.52	0.98	0.9991	4.2	3.0	4.5	4.2	1.7	2.2	91.4	100.3	95.9
Acrylamide	3.94	193	0.08	0.24	0.9995	5.8	2.5	2.6	5.0	1.1	0.9	111.3	95.8	99.5
Methacrylamide	8.30	196	0.23	0.69	0.9994	6.1	3.8	5.2	2.4	2.8	1.9	91.2	93.4	102.7
Thiourea*	2.56	232	–	–	–	–	–	–	–	–	–	–	–	–

* used as the internal standard for the standard addition calibration form LOQ to 10 μg/mL. RT, retention time; LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation; L-QC, low-level quality control substance at 1 μg/mL; M-QC, mid-level quality control substance at 2 μg/mL; H-QC,, high-level quality control substance at 5 μg/mL.

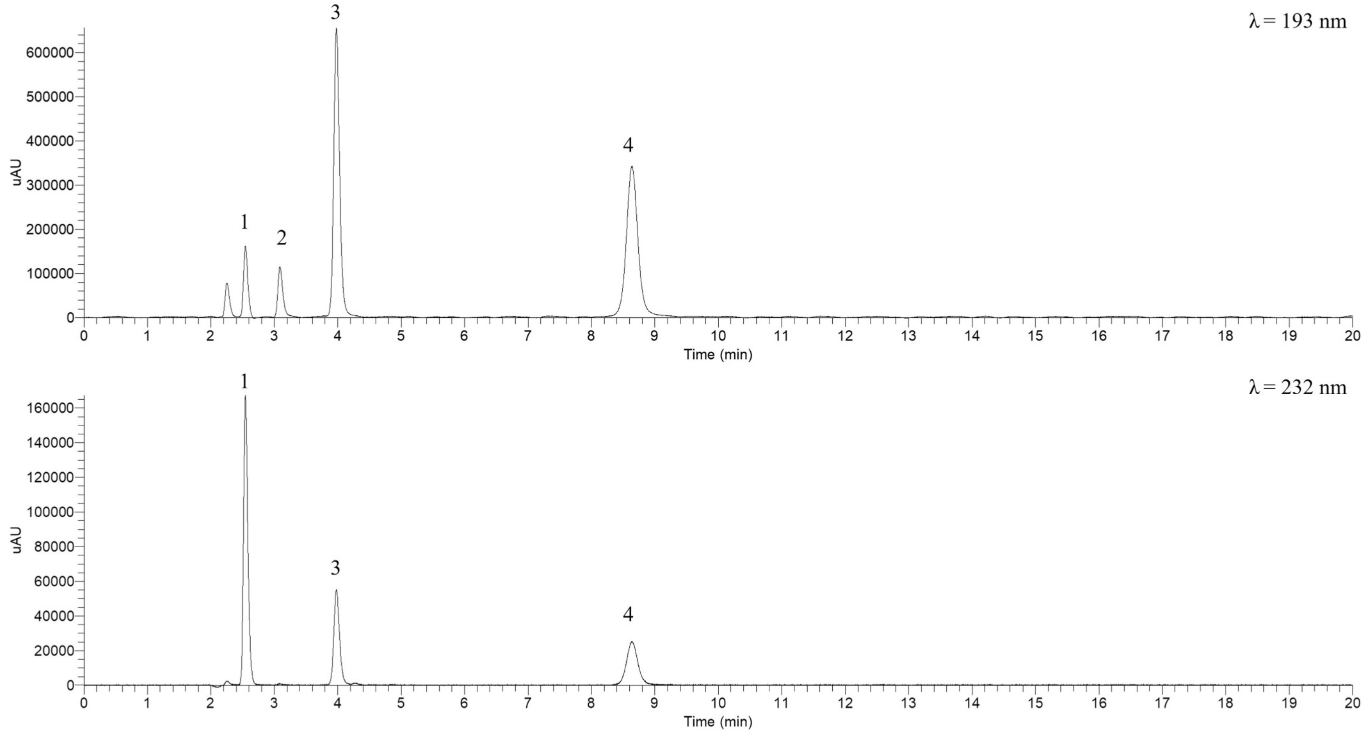

Fig. 6. HPLC-UV Chromatogram of non-roasted almond milk enriched with 1 μg/ml Thiourea (1, Internal Standard), Glycidamide (2), Acrylamide (3), and Methacrylamide (4), monitored at their UV–Vis maxima.

H-QC, respectively. For AA, recoveries were 111.3 %, 95.8 %, and 99.5 %, and for MA, 91.2 %, 93.4 %, and 102.7 %, demonstrating the method's reliability for accurate quantification (Table 1).

Matrix effects were assessed by comparing the slope of the external calibration with standard addition calibration curves (Table S1). The results indicated some degree of ion suppression (for GA) or enhancement, particularly for MA (220.2 %) and AA (148.7 %). These findings highlight the importance of using standard addition calibration with an internal standard to ensure robustness.

To verify the applicability of the method to the full range of PBBs analyzed, the products were classified using the AOAC food matrix triangle based on their macronutrient distribution (Fig. S5), according to the nutritional information provided on their labels. Most of the samples clustered within the lower-left area of the triangle, indicating balanced compositions of proteins, carbohydrates, and fats. According to U.S. Food and Drug Administration (2019) guidelines, matrix extension studies are not required when samples fall within a similar macronutrient region, supporting the applicability of the method to the complete set without the need for further matrix evaluations. Moreover, the control charts obtained for the QC samples at the three concentration levels (Fig. S6) remained within the established warning and decision limits throughout the analytical runs. No systematic trends or out-of-control points were observed, demonstrating the stability of the analytical system and supporting both the robustness of the method and the acceptability of the daily analytical sequence.

Table S2 compares analytical methods published within the last five years for the determination of AA. Most methods use HPLC-MS/MS for AA determination, or for AA simultaneously with other processing contaminants, although HPLC-UV and GC–MS have also been explored. In this regard, the proposed method achieves comparable or even lower LOQs than other HPLC-UV approaches, such as the one described by Altunay et al. (2023), and delivers adequate sensitivity for the target application. While MS-based methods report lower detection limits, they are generally restricted to AA alone or require more complex sample preparation workflows. Only one recent study (Custodio-Mendoza et al., 2024) describes the simultaneous quantification of AA and GA, although applied to infant formula and using GC–MS. It is worth noting that the simultaneous determination of AA and its polar metabolites poses a chromatographic challenge due to acrylamide's low retention on C18 reversed-phase columns, as previously reported by Ikhsan et al. (2022). This limitation often leads to the development of targeted methods optimized exclusively for acrylamide or its precursors and limits the scope of simultaneous quantification.

Despite this analytical complexity, the proposed method successfully achieves baseline separation of AA, GA, and MA and although higher than the LOQs reported for MS-based methods (as low as 1.5 ng/mL in Ciesarová et al., 2021), they remain appropriate for the concentration levels typically found in PBBs as previously reported by Pucci et al. (2024). It should be noted that GC–MS methods involving xanthydrol derivatization can also reach lower detection limits than those reported here such as in the determination of AA, GA and acetamide in infant formulae (Custodio-Mendoza et al., 2024). In terms of reproducibility and accuracy, the proposed method performs at a level comparable to or better than previously published methods. Reported precision and recovery values are better or similar to with those obtained using MS-based approaches (Cheng et al., 2021; Feng et al., 2023; González-Gómez et al., 2021; Pogurschi et al., 2021; Oellig et al., 2022; Kumari et al., 2023; Sun et al., 2022; Della Posta et al., 2025), and are within the range or superior to those described for UV-based methods (Altunay et al., 2023, Deribew & Woldegiorgis, 2021, Verma & Yadav, 2022; Della Posta et al., 2025), confirming the method's reliability for quantitative analysis in complex matrices.

3.3. *Occurrence of acrylamide, Glycidamide, and Methacrylamide in plant-based beverages*

The optimized validated µ-dSPE-HPLC-PDA methodology was applied to determine the levels of AA, GA, and MA in the 15 analyzed PBBs and the results are summarized in Table 2. Neither GA nor MA were determined in any of the analyzed samples, while AA was quantified in 14 of the samples, with the exception of the non-roasted almond beverage. A descriptive analysis of AA content among positive samples (Table S3) revealed a relatively narrow range of values, with a mean concentration of 0.81 µg/mL and a maximum of 1.17 µg/mL. The Kruskal-Wallis test did not indicate significant differences in AA content across the sample groups ($\chi^2 = 13$, df $= 13$, $p = 0.448$), and Dunn's post hoc comparisons similarly showed no statistically significant differences between specific sample pairs. Furthermore, correlation analysis indicated no significant association between labelled protein content and AA content, as evidenced by both the Pearson ($r = -0.038$, $p = 0.898$) and Spearman ($\rho = 0.084$, $p = 0.776$) correlation tests, suggesting that protein concentration was not a primary determinant of AA formation in the analyzed samples.

Despite the lack of statistically significant differences, some trends were observed in the dataset. AA levels were slightly higher in oat-based samples compared to other matrices, as illustrated in the boxplot (Fig. 7). Mixed protein beverages also exhibited relatively elevated AA concentrations, with the highest values detected in the 15 % oat - 1.2 % hazelnut beverage. Conversely, the lowest AA concentrations were found in rice-based beverages (Fig. S7), aligning with previous reports.

Table 2
Occurrence of Glycidamide, Acrylamide, and in Plant-Based Beverages.

Sample	Glycidamide		Acrylamide		Methacrylamide	
	µg/mL	±SD	µg/mL	±SD	µg/mL	±SD
Non roasted Almond 5 %	ND	–	ND	–	ND	–
Roasted Almond 2.3 %	ND	–	0.93	0.05	ND	–
Roasted Almond 4 %	ND	–	1.02	0.02	ND	–
Coconut 8.5 %	ND	–	0.43	0.06	ND	–
Coconut 8.6 %	ND	–	0.56	0.16	ND	–
Coconut 5.7 % + Soy 2.9 %	ND	–	0.96	0.08	ND	–
Coconut 3.5 % + Oat 12 %	ND	–	0.91	0.02	ND	–
Rice 16 %	ND	–	0.33	0.07	ND	–
Rice 10 %	ND	–	0.55	0.06	ND	–
Oat 15 %	ND	–	1.17	0.03	ND	–
Oat 13 %	ND	–	1.04	0.03	ND	–
Oat 8.7 %	ND	–	0.79	0.10	ND	–
Oat 10 %	ND	–	0.81	0.11	ND	–
Oat 10.3 %	ND	–	0.82	0.07	ND	–
Oat 15 % hazelnut 1.2 %	ND	–	1.03	0.21	ND	–

SD, standard deviation; ND, non-determined; <LOQ, determined at a concentration below the limit of quantification.

Pucci et al. (2024) similarly noted higher AA levels in oat-based drinks and lower levels in rice-based samples, suggesting that raw material composition plays a significant role in AA formation in PBBs. However, the AA concentrations reported by Pucci et al. were generally lower than those observed in the present study, potentially due to the lower protein content of their analyzed beverages, which ranged from 0.5 % to 3.5 % for oat and 0.2 % to 0.6 % for rice. Additionally, Pucci et al. (2024) emphasized that AA is typically not formed in substantial amounts in foods with high water content, such as PBBs, but rather results from thermal treatments applied to raw materials, such as roasting. This observation aligns with the current findings, where no AA was detected in the non-roasted almond sample, underscoring the potential impact of raw material processing on AA levels.

To validate these findings, further studies involving a larger sample set with diverse matrices and processing conditions would be necessary to confirm the observed trends and elucidate the influence of raw material composition and thermal treatment on AA formation in PBBs.

The absence of GA and MA in all samples analyzed deserves specific consideration. GA is mainly formed in vivo through the epoxidation of acrylamide catalyzed by cytochrome P450 2E1, rather than during food processing (Von Tungeln et al., 2012; Favor & Shelby, 2005). Consequently, its occurrence in foods is generally very limited or below typical detection limits for LC-based methods (Custodio-Mendoza et al., 2024). MA has been identified mainly in food-contact materials, especially in paper-based packaging, as a residual or degradation product of polyacrylamide and methacrylate copolymers used as paper-reinforcing or surface-treatment agents (Zhang et al., 2016). These compounds can migrate into food only under specific conditions, such as contact with aqueous or alcoholic food simulants, elevated temperature, or prolonged storage. Since all plant-based beverages examined here were UHT-treated and packaged in multilayer cartons free from methacrylic polymers, migration pathways for MA were not present. Moreover, the limits of detection (0.08–0.52 µg/mL) and LLOQ values (0.24–0.98 µg/mL) achieved in this study were sufficiently low to quantify these analytes if present at concentrations reported in other thermally processed foods (Custodio-Mendoza et al., 2024; Zhang et al., 2016). Therefore, the absence of GA and MA most likely reflects their true non-occurrence rather than analytical limitations. The aqueous, low-fat, and mildly processed nature of PBBs further reduces precursor availability and promotes dilution or degradation of reactive intermediates, explaining why only acrylamide, formed through the Maillard reaction between reducing sugars and asparagine, was detected.

3.4. *Sustainability and practical applicability assessment of the µ-dSPE method*

In addition to analytical performance, the sustainability of the sample preparation procedure was evaluated using the AGREEprep metric (Fig. 8), which considers ten criteria related to environmental impact, safety, and operational efficiency (Pena-Pereira et al., 2022). The method received the highest score for the absence of hazardous reagents (1.0), reflecting its suitability for routine analysis without compromising laboratory safety. In the AGREEprep framework, instrument selection is also an integral component of sustainability (Criterion 9). Although MS-based techniques offer superior sensitivity, they are penalized for high energy consumption, extensive maintenance, and limited accessibility, which shift the environmental burden from sample preparation to detection (Pena-Pereira et al., 2022). In contrast, the use of HPLC-PDA represents a greener post-sample-treatment configuration, favoring simplicity, lower energy demand, and broader applicability in routine food-control laboratories (Wejnerowska & Narloch, 2023). The method's performance (adequate for the typical concentration range of

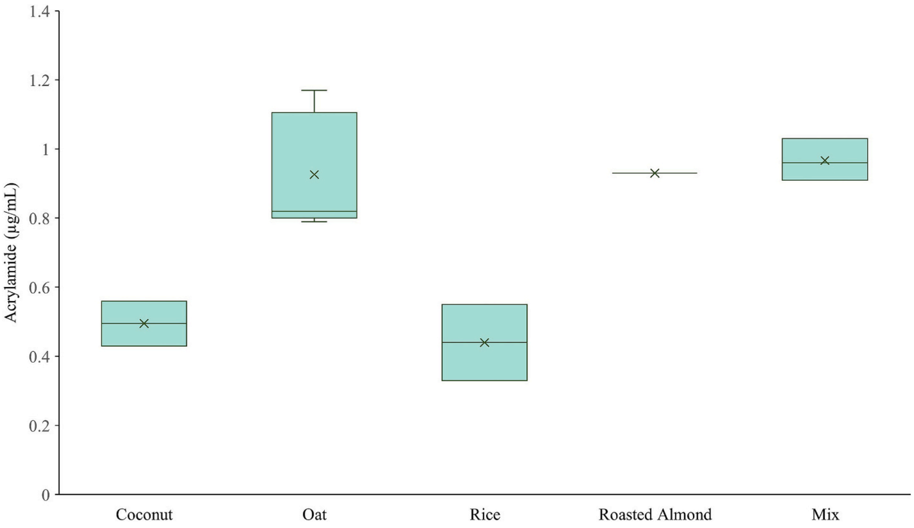

Fig. 7. Distribution of Acrylamide Content Across Different Protein Matrices in Plant-Based Beverages.

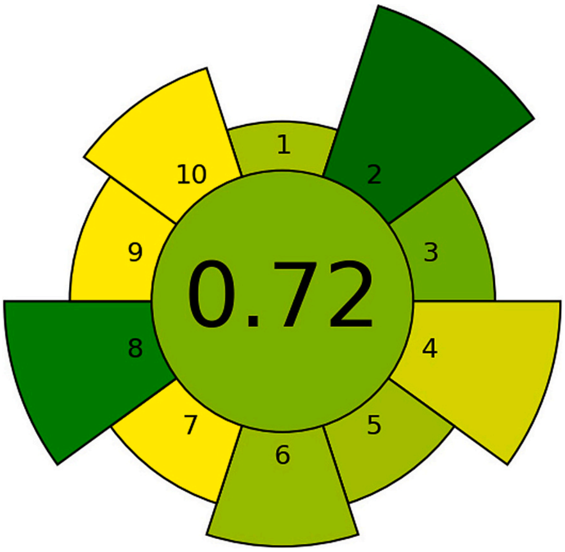

Fig. 8. Sustainability Assessment of the Method Using AGREEprep Tool. These criteria include favoring in situ sample preparation (1), using safer solvents and reagents (2), targeting sustainable, reusable, and renewable materials (3), minimizing waste (4), minimizing sample, chemical, and material amounts (5), maximizing sample throughput (6), integrating steps and promoting automation (7), minimizing energy consumption (8), choosing the greenest possible post-sample preparation configuration for analysis (9), and ensuring safe procedures for the operator (10).

acrylamide in plant-based beverages) demonstrates that high sensitivity can be balanced with environmental responsibility and analytical sufficiency, aligning with AGREEprep's recommendation to prefer simple, widely available detection systems over sophisticated ones. It also performed well in terms of material sustainability (0.75) and energy consumption per analysis (0.94), supporting its alignment with green analytical chemistry principles. Moderate scores were observed for waste generation (0.59) and operator safety (0.50), the former likely related to the use of silica-based sorbent material (PSA) during the cleanup step. Nevertheless, the method maintains a favorable overall profile, with a limited number of preparation steps, good throughput (18 samples/h), and low energy demand, reinforcing its applicability as a cost-effective and environmentally responsible alternative for AA, GA and MA determination in PBBs.

The comparative AGREEprep assessment (Table S2) of analytical methods developed in the last five years (2021–2025) for the determination of acrylamide and related amides in food shows a clear trend toward miniaturization and solvent reduction, although most procedures still display low or moderate greenness (0.07–0.58). All methods were evaluated under equivalent conditions, assuming the same model of equipment and the default weighting for throughput and energy parameters. The parameter related to the use of hazardous or problematic materials remains critical: most applications employ large volumes of organic solvents, particularly hexane and halogenated media, that substantially penalize their scores. In this respect, Altunay et al. (2023) achieved a comparatively better performance due to the smaller solvent volumes required for the preparation of their alkanol-based supramolecular solvent, while Verma and Yadav (2022), despite implementing Carrez clarification, reached only an average score in this category. Another persistent weakness across studies concerns the sustainability, renewability, and reusability of materials. In most cases, the proportion of renewable components is low, which reduces the overall sustainability of the process. Exceptions are Altunay et al. (2023), Della Posta et al. (2025), and Custodio-Mendoza et al. (2024), where at least half of the reagents and materials were renewable, although single-use, thus attaining the highest values in this parameter. Waste generation also remains problematic, with only Oellig et al. (2022) reporting negligible residues, followed by Altunay et al. (2023), whose aqueous waste was below 1 mL per sample. In terms of sample economy, the approaches by Pucci et al. (2024), Altunay et al. (2023), Della Posta et al. (2025), and Mesías et al. (2023) stand out for their reduced sample masses and volumes, confirming the benefits of microscale strategies. Throughput values are likewise higher in Altunay et al. (2023), whose vortex-assisted DLLME enables up to twelve simultaneous extractions, and in Pucci et al. (2021) and Mesías et al. (2023), who used SPE manifolds with similar capacity. Integration and automation continue to represent a limitation, as most procedures remain essentially manual or, at best, semi-automatic. Energy consumption is high in nearly all methods because of the combined operation of ultrasound baths, centrifuges, evaporators, and heating systems. Only Custodio-Mendoza et al. (2024) and Altunay et al. (2023) significantly reduced their energy footprint thanks to their microextraction design, the latter requiring barely 2–3 Wh per sample. Another penalizing factor in AGREEprep is the environmental burden of the final determination, since LC- or GC–MS systems, although indispensable for sensitivity, lower the sustainability score (Gao et al., 2021). UV-based detection yields intermediate results, while the spectrophotometric approach of Altunay et al. (2023) achieved the highest score due to its minimal instrumental and solvent requirements. Finally, most applications still involve at least two GHS hazard categories (typically flammable and irritant substances) keeping the overall safety performance at a low to medium level. Altogether, these results confirm that despite recent progress, the green potential of acrylamide analysis methods remains limited. Compared with recent methods (2021–2025), our procedure achieves the highest AGREEprep score (0.72), standing at the greenest end of current practice. It surpasses most existing protocols that rely on large solvent volumes or energy-intensive steps, offering excellent safety, low energy demand, and a balanced material profile with around half of the reagents being renewable or low-impact. Waste generation remains moderate but clearly below that of conventional LLE or SPE approaches, and sample consumption and throughput are within the best-performing range reported in the literature. While automation is still limited, the combination of a simplified workflow and an HPLC-PDA configuration (greener than typical LC/GC–MS systems) positions this method as a sustainable and analytically efficient alternative for the determination of acrylamide and related amides in foods.

Equally, the practical applicability of the developed μ-dSPE–HPLC-PDA method was evaluated using the Blue Applicability Grade Index (BAGI) (Manousi et al., 2023). This metric assesses ten operational and instrumental criteria, such as analytical scope, throughput, automation, reagent accessibility, and sample requirements, to determine a method's readiness for routine implementation in laboratory settings. The present method achieved high scores across all categories (Fig. 9), reflecting its quantitative and confirmatory character, multi-analyte capability, and reliance on simple, widely available instrumentation. The use of miniaturized sample preparation and semi-automated injection (HPLC autosampler) ensures a throughput exceeding 10 samples h^{-1}, while the use of common reagents and small sample volumes (< 10 mL) further supports operational efficiency. The BAGI profile therefore confirms that the proposed method combines environmental sustainability with excellent practical feasibility, meeting the principles of White Analytical Chemistry by integrating efficiency, reliability, and accessibility in a single analytical workflow.

Fig. 9. Practical Applicability Assessment of the Method Using the Blue Applicability Grade Index (BAGI) Tool. The ten BAGI criteria evaluate method feasibility and operational performance: (1) type of analysis, (2) multi- or single-element capability, (3) analytical technique, (4) simultaneous sample preparation capacity, (5) sample preparation approach, (6) sample throughput (samples h^{-1}), (7) reagents and materials, (8) preconcentration requirements, (9) degree of automation, and (10) sample amount required. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Conclusion

A novel, rapid, and environmentally friendly μ-dSPE method combined with HPLC-PDA was successfully developed, optimized, and validated for the simultaneous determination of AA, GA, and MA in PBBs. To the best of our knowledge, this is the first method specifically designed and validated for this matrix type capable of simultaneously quantifying the three analytes without derivatization, and the first to introduce Th as an internal standard for this application. Unlike conventional μ-dSPE/QuEChERS and many MS-based workflows that rely on organic solvents and/or derivatization and often presuppose isotopically labelled IS for reliable quantification, our procedure operates in an entirely aqueous medium with direct UV detection, thereby simplifying operations and reducing chemical hazards and waste, in alignment with green analytical chemistry.

Method optimization through multivariate design identified PSA as the most effective clean-up sorbent and demonstrated that both sorbent composition and quantity significantly affect clean-up efficiency and analyte recovery. The final protocol exhibited excellent linearity, accuracy, and precision, fully complying with FDA validation guidelines, and proved applicable to a diverse array of commercial PBBs—including almond, oat, coconut, rice, and mixed formulations—without the need for matrix extension studies. Furthermore, the method meets green chemistry principles, as evidenced by its high AGREEprep score (0.72), driven by the elimination of hazardous reagents, low energy consumption, and minimal waste generation.

AA was detected in 14 out of the 15 analyzed samples, with the exception of the non-roasted almond beverage, whereas GA and MA were not detected in any sample. Oat-based samples exhibited slightly higher AA levels compared to other matrices, with the highest concentrations observed in the 15 % oat - 1.2 % hazelnut beverage, while rice-based samples consistently presented the lowest AA concentrations. Despite these observed trends, statistical analysis did not reveal significant differences in AA content across matrices, nor a significant correlation between labelled protein content and AA levels. These findings align with previous reports suggesting that AA formation in PBBs is more likely associated with thermal processing of raw materials than with protein content or beverage formulation. Expanding the sample set to include a wider range of processing conditions and matrices would further substantiate these observations and clarify the role of raw material composition in AA formation in PBBs.

With its simplicity, rapidity (20 min total preparation), cost-efficiency, and accessibility—requiring only standard laboratory equipment—this method holds promise not only for routine monitoring and quality control in the food industry but also for broader applications. With appropriate matrix extension studies, it could be effectively adapted to other aqueous and protein-rich food systems, such as broths, plant-based yogurts, and infant formulae, providing a valuable tool for the surveillance of process-related contaminants in health-oriented and emerging food products. Ultimately, the application of this method in routine analysis could contribute to a deeper understanding of AA formation mechanisms in complex matrices, guiding mitigation strategies and improving food safety monitoring.

CRediT authorship contribution statement

Jorge Antonio Custodio-Mendoza: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Agata Antoniewska-Krzeska:** Visualization, Validation, Investigation, Formal analysis. **Patryk Pokorski:** Visualization, Validation, Investigation, Formal analysis. **Havva Aktaş:** Visualization, Validation, Investigation. **Marcin Kurek:** Writing – review & editing, Visualization. **Flavio A. Franchina:** Writing – review & editing, Visualization. **Rui Miguel Ramos:** Writing – review & editing, Visualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jorge Antonio Custodio Mendoza reports financial support was provided by National Science Centre Poland. Jorge Antonio Custodio Mendoza reports a relationship with Spain Ministry of Science and Innovation that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The funders had no role in the study's design, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2025.147261.

Data availability

Data will be made available on request.

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