

Article

Development and Validation of an HPLC-DAD Method to Determine Alkylphenols in Milk

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Abstract: While providing considerable societal and economic benefits, plastic packaging leads to global pollution and poses health risks. Plastic additives like alkylphenols (APs) can interfere with endocrine functions even at low concentrations. Therefore, developing and validating analytical methods for their routine dosage in foods is paramount. The present work validated a chromatographic method to quantify alkylphenols (4-tert-octylphenol, 4-n-octylphenol mono-ethoxylate, 4-n-octylphenol, and 4-n-nonylphenol) in milk. The analytical method uses Chem Elut S a rapid supported liquid extraction (SLE) cartridges to eliminate the matrix effect, and reverse phase chromatography linked to a Diode Array Detector (DAD) to dosage the alkylphenols. The method was validated using the strategy of accuracy profiling, a decision-making instrument that calculates the method's total error, encompassing bias and standard deviation. The reliability of the test was defined by the lack at the retention times of the APs of interfering peaks, the close linear relationship between the independent and the dependent variables in the regression model, the excellent precision at each concentration level for intra-day and inter-day measurements, and the errors of the procedure (systematic and random) estimated within the pre-established acceptability limits ($\pm 10\%$). The minimal environmental impact and ease of execution suggest its use in routine analyses.

Keywords: alkylphenols residue analysis; food safety; food quality; 4-tert-octylphenol; 4-n-octylphenol mono-ethoxylate; 4-n-octylphenol; 4-n-nonylphenol; ecofriendly analytical method

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1. Introduction

Milk is a key component of the global diet, as it provides protein, calcium, and vitamins A, D, B2, and B12 [1]. It is often sold in plastic containers. Unfortunately, plastic can leach over 13,000 different chemicals into food, which can lead to serious health concerns [2]. These chemicals include alkylphenols (APs), bisphenols, per- and poly-fluoroalkyl substances, phthalates, polycyclic aromatic hydrocarbons, neonicotinoids, as well as various metals and metalloids [3–5]. Due to its fat content, milk is particularly susceptible to contamination from lipophilic pollutants, such as APs, phthalates, pesticides, and antibiotics, that can migrate from packaging [6]. APs are organic compounds characterized by a phenolic ring and an alkyl chain, whose length of the chain, denoted as “n”, varies from 1 to 12 [7]. They play a key role in the production of various materials, including plastics like high-density polyethylene (HDPE), polyvinyl chloride (PVC), and polyethylene terephthalate (PET) [8], as well as in the manufacturing of paper, textiles, and agricultural chemical products [9]. The APs are also the main degradation products of alkylphenol ethoxylates, non-ionic surfactants employed as detergents, dispersants, or solubilizers [10].

4-octylphenols (4-OP) and 4-nonylphenols (4-NP) account for over 80% of the total production of alkylphenols [11]. The APs are classified as endocrine-disrupting chemicals (EDCs) because they interfere with the production, release, transport, activity, and removal of natural hormones that are crucial for maintaining homeostasis, reproduction, and development [12]. They have a structural similarity to natural estrogens [13], which allows them to trigger early onset puberty [14], impact steroidogenesis and folliculogenesis [15], reduce semen quality, cause infertility, alter fetal development, result in complications during pregnancy, lead to genital malformations, and increase the risk of cancer [16]. Polymer-based packaging can leach APs into fatty foods [17], a risk that is especially notable when milk is kept in plastic containers. This type of contamination represents a danger to individuals of all ages worldwide, highlighting the issue's urgency. The European Commission, in Regulation EU 10/2011 and its amendments, established strict migration limits for materials and objects in contact with food [18] to safeguard the health and safety of consumers. Measuring APs in milk is difficult due to their low concentration and the marked matrix effect due to lipids and proteins [19]. Several tests are described in the literature that allow the APs dosage. They include a pretreatment phase to remove the matrix interferents and subsequent dosage of the APs. Pretreatment techniques include Easy, Cheap, Effective, Rugged, and Safe (QuEChERS), solid-phase extraction (SPE), solid-phase microextraction (SPME), magnetic solid-phase microextraction (MSPE), and liquid-phase microextraction (LPME).

The QuEChERS methodology is simple, affordable, and highly effective [20,21]. The SPE separates compounds based on their partition coefficients between solid and liquid phases [22]. The SPME is a solvent-free method that facilitates the partitioning of analytes between a coated adsorbent and the sample matrix. It is followed by desorption into an appropriate mobile phase [23]. The MSPE is a variant of SPE that utilizes magnetic adsorbents to capture target analytes [24]. The LPME encompasses two-phase and three-phase methods, including single-drop microextraction (SDME) [25], dispersed LPME (DLLME), and hollow-fiber LPME (HF-LPME) [26].

The dosage of APs can be achieved through chemical analyses by using chromatographic linked with spectroscopic apparatus like gas chromatography–mass spectrometry (GC-MS), liquid chromatography–mass spectrometry (LC-MS), liquid chromatography–tandem mass spectrometry (LC-MS/MS), and biological methods like enzyme-linked immunosorbent assay (ELISA) [19].

GC-MS offers high selectivity, sensitivity, and reproducibility but requires complex derivatization processes [27]. LC-MS/MS gives high sensitivity, selectivity, throughput, and the ability to conduct multiple analyses simultaneously, but it is costly [28,29].

ELISA is an immunoassay with relatively low sensitivity [30].

The lack of official analytical methods and the absence of validated techniques that yield results with known accuracy and precision pose significant challenges in determining the extent of alkylphenols' contamination in milk samples, thereby impacting consumer safety.

This study proposed a chemical analytical method to quantify four alkylphenols selected from those that most frequently pollute food matrices [11] (4-tert-octylphenol, 4-n-octyl phenol mono-ethoxylate, 4-n-octyl phenol, and 4-n-nonylphenol) (Figure 1) in milk. It consists of a one-step rapid cleanup process (Chem Elut S cartridge) and a cost-effective chromatography technique (reverse phase HPLC-DAD) dosage.

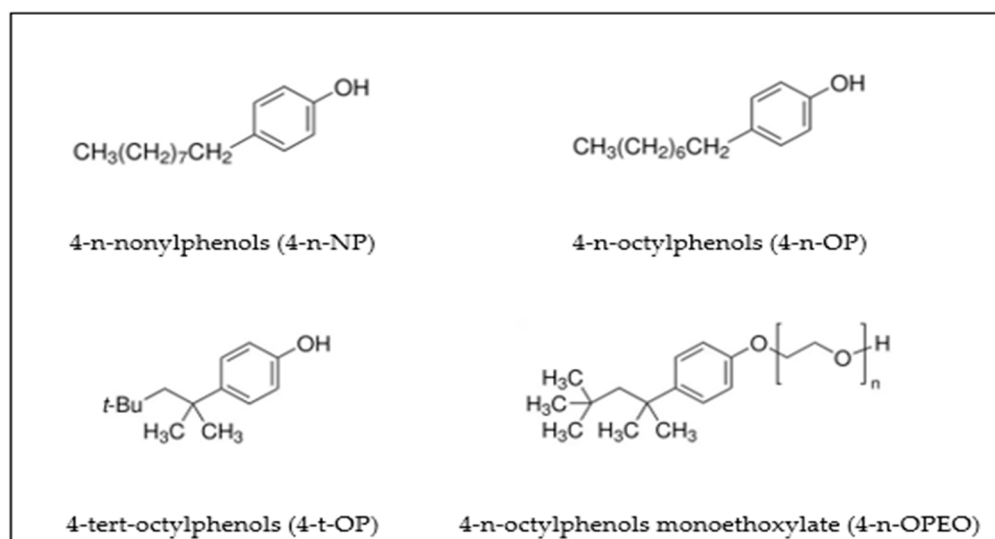


Figure 1. Alkylphenols checked in fresh whole milk with a lipid percentage content of no less than 3% packaged in plastic bottles.

Previous research to extract contaminants from milk has exploited diatomaceous earth cartridges' high porosity and aqueous adsorption capacity. They can prevent emulsion formation and promote interaction with organic solvents [31]. This study used the SLE Chem Elut S cartridges filled with a synthetic inert porous adsorbent for the first time. The regular particle size of the adsorbent allows elevated testing efficiency, ensures consistent flow and uniformity across all batches, and minimizes variability among analysts and batches, overcoming some disadvantages of using diatomaceous earth in SLE. In Chem Elut S, the sample is placed into an SLE cartridge. Following this, an immiscible organic solvent is applied over the synthetic sorbent to elute the target analytes while eliminating unwanted matrix interferences. The Chem Elut S cartridges allow rapid analysis, even for those with limited experience, necessitate a minimal volume of extraction solvent, diminish matrix interferences, and allow concentration of the analytes of interest. They enable reliable and simple analytical processes, can function independently, and require only the mobile phase selection.

Concerning the APs dosage, an HPLC equipped with a DAD detector was employed. The DAD detector is less selective and sensitive than a mass spectrometer but has lower purchase and maintenance costs than the MS apparatus. Therefore, it is widely accessible and commonly available in commodity laboratories, ensuring the test's broader adoption for routine analyses. Afterward, the proposed methodology underwent a thorough validation process to guarantee the accuracy and reliability of the results achieved. Metrological quality in xenobiotics analysis must balance consumer safety and producer risks. Laboratories adhering to ISO 17025 standards [32] must validate methods and assess their uncertainties to confirm the credibility of their results [33]. For this purpose, the Société Française des Sciences et Techniques Pharmaceutiques (SFSTP) has proposed the "accuracy profile" strategy for the validation of analytical methods. This strategy is based on the β -expectation tolerance intervals of the total error concept and supports the ICH Q2 guideline [34–36]. The accuracy profile approach has proven effective in pesticide analyses. Notable examples include detecting aflatoxins in almonds [37], neonicotinoids in wheat [3] and Moroccan spearmint [33], and glyphosate and glufosinate in various foods [38], as well as quantifying furan in apple puree and infant formula [39].

The aim of this work was to develop and validate a procedure usable in commodity laboratories (simple, rapid, and capable of providing reliable results) for routine analysis of alkylphenols in milk, pending the establishment of official methods for this purpose.

2. Materials and Methods

2.1. Solvents

Acetonitrile (CAS-n 75-05-8; purity $\geq 99.9\%$), acetic acid (CAS-n 64-19-7; purity $\geq 99.9\%$), and dichloromethane (CAS-n 75-09-2; purity $\geq 99.8\%$) for HPLC were purchased from Carlo Erba Reagents (Carlo Erba Reagents, Cornaredo, MI, Italy).

Water for HPLC was obtained by distilling and vacuum filtering water on Millipore filters (Millipore HA WP 04700; Merck, Burlington, MA, USA).

2.2. Analytical Standards

4-tert-octylphenol (4-t-OP; CAS Number 140-66-9 purity 97%), 4-n-octylphenol (4-n-OP; CAS Number 1806-26-4; purity 98.5%), and 4-n-nonylphenol (4-n-NP; CAS Number 104-40-5; purity $\geq 98.0\%$) were bought by Merck (Merck; Darmstadt, Germany). 4-n-octylphenol mono-ethoxylate (4-n-OPEO; CAS Number 2315-67-5; >purity 95%) was bought by the LGC group (LGC group; Teddington, Middlesex, UK).

2.3. Apparatus

Technical scale balance Gibertini TM2000d: 0.1 g was bought from Gibertini (Gibertini, Novate Milanese (MI), Italy).

Analytical balance Gibertini E42S d: 0.1 mg was purchased from Gibertini (Gibertini, Novate Milanese (MI), Italy).

Ultrasound Branson 2210 BV was bought from Branson (Branson, Cinisello Balsamo (MI), Italy).

Rotary vacuum evaporator Buchi 461 was obtained from Buchi (Buchi, Flawil, Swiss).

HPLC system Thermo Finnigan[®] P4000 was purchased by Thermo Fisher Scientific (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a four-way in-line degasser, double reciprocating piston quaternary pump, injector Rheodyne model 7125, and UV 6000 LP photodiode detector (Thermo Fisher Scientific, Waltham, MA, USA).

Chem Elut S Agilent cartridges were obtained from Agilent (Agilent, Santa Clara, CA, USA).

2.4. Sample

Twelve fresh whole milk with a lipid percentage content of no less than 3% packaged in plastic bottles selected from the top brands in Italy were purchased from local supermarkets in Naples, Italy, in 2023. All samples were kept under cold-chain conditions.

2.5. Sample Extraction and Cleanup with Chem Elut S Cartridge

A milk sample (5 g) was loaded onto the cartridge. The sample was left to adsorb on the solid phase for 15 min to allow a homogeneous distribution of the cartridge filling material. A needle with a Luer lock (0.7×30 mm) was connected to the cartridge outlet as a flow restrictor. Successively, the sample was eluted with a slow and constant flow of dichloromethane (5 mL, 3 times). The extraction solvent was removed using rotavapor, and the dry residue was taken up with 90/10 acetonitrile/water at 0.01% (*v/v*) in acetic acid pH 4.02 (1 mL) and subsequently subjected to analysis by HPLC.

2.6. HPLC (High-Performance Liquid Chromatography) Chromatography's Parameters

HPLC experiments were performed on an HPLC Thermo Finnigan[®] P4000 system interfaced to a Diode Array Detector, setting the wavelength at 278 nm. Data acquisition and analysis were operated using the standard software Agilent Chemstation Rev. B.02.01[®] (Agilent, Santa Clara, CA, USA). The analytical column was an octadecylsilyl (ODS) Hypersil 5 μ (125 mm $\times$ 4 mm ID, 5 μ m particle size) from Thermo Fisher Scientific (Thermo

Fisher Scientific, Waltham, MA, USA). A mixture of 90/10 acetonitrile/water with 0.01% (*v/v*) acetic acid (pH 4.02) was employed as the mobile phase. The injection volume was 20 μ L, and the flow rate was 1 mL/min.

2.7. Method Validation

The test was validated according to the French Society of Pharmaceutical Sciences and Techniques (SFSTP) guidelines, applying the total error approach (accuracy profile) [33–36]. The predefined acceptance limit was $\pm 10\%$, and the β -expectation tolerance interval was 95%.

2.7.1. Experimental Designs

Calibration and validation designs were based on 3 days ($k = 3$), 3 replicates ($n = 3$) with 5 concentration levels ($m = 5$) for calibration standards, and 3 concentration levels ($m = 3$) for validation standards. Validation and calibration measurements were collected on the same days.

2.7.2. Calibration Standards

A stock solution of each analyte (1 mg/mL) was prepared for each AP by dissolving 50 mg of each analyte in 50 mL of acetonitrile. Calibration standards were prepared by diluting a standard multicomponent solution containing the four alkylphenols (10 μ g/mL) to the final concentration of 0.10, 0.25, 0.50, 1.0, and 2.0 μ g/mL.

Matrix-matched calibration curves, using blank matrix extracts, were prepared at the same concentration levels to study the matrix effect in terms of the influence of interfering compounds on the analytes' quantification.

2.7.3. Validation Standards

Validation samples were obtained by spiking a milk sample (5 g, resulting negative in the analysis, considered a matrix blank) with convenient volumes of the multicomponent solution of the 4 alkylphenols to obtain three concentration levels: 0.1, 1.0, and 2.0 mg/Kg for each analyte.

2.7.4. Matrix Effect

The matrix effect, expressed as relative standard deviation (%), was investigated by comparing standards in solvent (chromatographic mobile phase) with matrix-matched standards.

$$ME(\%) = \frac{\text{Slope solvent}}{\text{Slope matrix}} \times 100$$

2.7.5. Limits of Detection (LOD) and Quantification (LOQ)

The values of LOD and LOQ were calculated from ordinary least-squares regression data. LOQ was obtained by multiplying 10 times the standard deviation of the intercept and 3 times the standard deviation of the intercept to calculate the LOD [40].

2.7.6. Trueness

The systematic errors were estimated as Bias%.

$$\text{Bias}\% = \frac{X_{\text{meas}} - X_{\text{exp}}}{X_{\text{exp}}} \times 100$$

X_{meas} = measured value

X_{exp} = expected value

2.7.7. Precision

The method's relative errors were estimated as Relative standard deviation % (RSD%).

$$\text{RSD}\% = \frac{S * 100}{\bar{x}}$$

S = sample standard deviation

$\bar{x}$ = sample mean

2.7.8. Uncertainty

The relative expanded uncertainty (%) was calculated by dividing the expanded uncertainty by the corresponding concentration and utilizing a coverage factor of $k = 2$, which indicates an interval surrounding the results within which the unknown true value can be identified with a confidence level of 95%.

2.8. Statistical Analysis

Microsoft Office Excel 2010 (Microsoft, Redmond, WA, USA) was used to perform statistical analyses.

3. Results

3.1. Cleanup Procedure Optimization

A sample devoid of APs was supplemented with APs. The APs' recoveries obtained utilizing various solvents (ethanol, ethyl acetate, acetonitrile, acetone, dichloromethane) were assessed to identify the best eluent for extraction, able to ensure compatibility with the volumes and recovery rates. Of all the solvents tested, dichloromethane exhibited the best yields (>70%) and minor matrix interference.

The APs' retention times were reported in Figure 2: 4-*tert*-OP (t_R 4.59), 4-*n*-OPEO₁ (t_R 4.82), 4-*n*-OP (t_R 5.63), 4-*n*-NP (t_R 6.64).

3.2. Method Validation

The accuracy profile approach was utilized to ensure the test's reliability. This method, rooted in rationality and statistical rigor, aligns seamlessly with ICH guidelines.

3.2.1. Linearity

The linearity of an analytical method refers to its capacity to yield results directly proportional to the analyte levels present in a sample within a specified range. The analytical procedure's linearity assessment was conducted across five calibration levels (0.10, 0.25, 0.50, 1.0, and 2.0 $\mu\text{g/mL}$), focusing on the correlation between the concentrations injected and the resulting areas. The lowest possible accuracy was derived from the calibration curve using ordinary least-squares linear regression that best fits the data such that the tolerance intervals fall within acceptable limits ($\pm\lambda$) for the working concentration range [41].

The results obtained for each APs in solvent and matrix ($R^2 > 0.9991$; residuals less than ± 5.3) indicate a good calibration function fit (Table 1).

3.2.2. Matrix Effect

The matrix effect for all APs tested in matrix and solvent was $\geq 99\%$.

The calibration data obtained for each alkylphenol in the matrix were shown in Table 1.

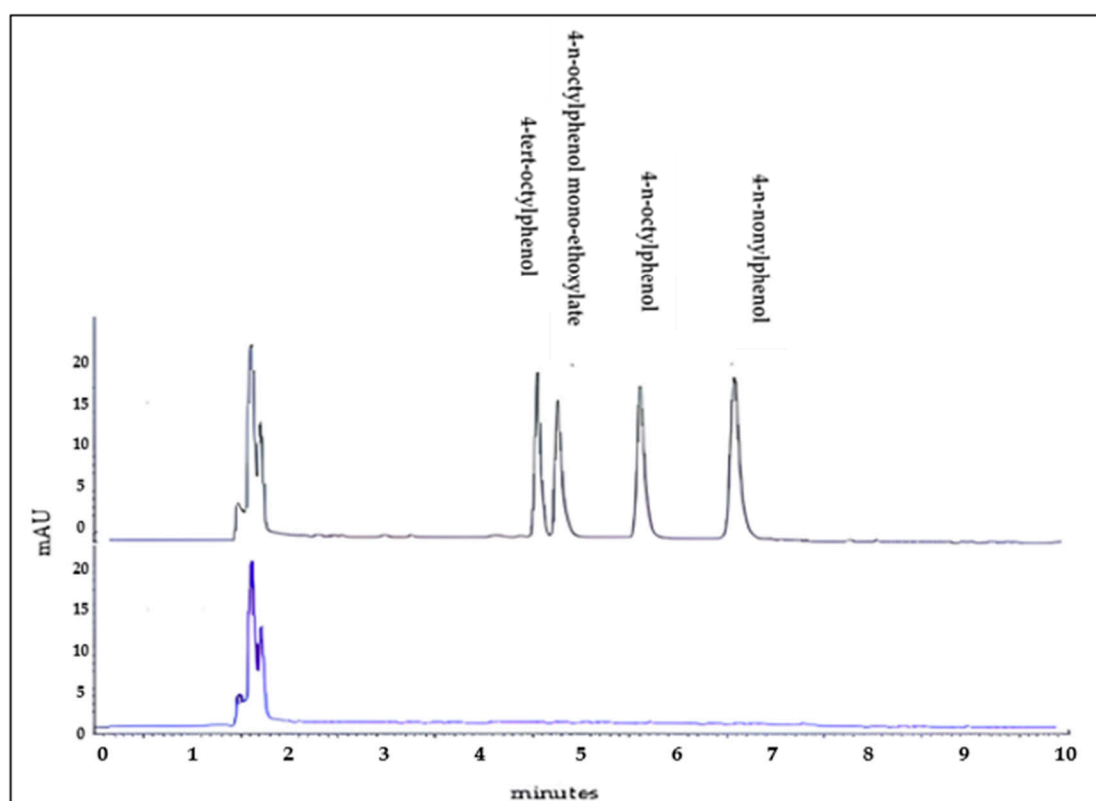


Figure 2. Blank's (milk sample without alkylphenols) HPLD-DAD chromatogram (blue line). Chromatogram of a milk sample enriched with 1.0 mg/Kg of a multicomponent solution containing the four alkylphenols examined (black line).

Table 1. Linear regression parameters obtained from the calibration curves for solvent calibration standards and matrix-matched calibration standards.

APs		Slope	Intercept	R ²	Residuals (%)	Matrix Effect
4-tert-OP	solvent	23,319 ± 0.001	618.5 ± 0.002	0.9997	±0.2	99.96
	matrix	23,328 ± 0.003	508.4 ± 0.003	0.9990	±1.7	
4-n-OPEO1	solvent	17,901 ± 0.002	−273.5 ± 0.001	0.9997	±5.3	99.92
	matrix	17,916 ± 0.003	−250.2 ± 0.003	0.9991	±3.5	
4-n-OP	solvent	27,065 ± 0.001	−355.5 ± 0.002	0.9996	±2.1	99.99
	matrix	27,068 ± 0.004	−346.8 ± 0.005	0.9994	±3.8	
4-n-NP	solvent	26,576 ± 0.002	133 ± 0.001	0.9998	±4.4	99.95
	matrix	26,588 ± 0.004	120 ± 0.002	0.9996	±1.8	

3.2.3. LOD and LOQ

The values of LOD and LOQ were calculated from ordinary least-squares regression data (Table 2).

Table 2. LOD and LOQ of APs.

APs	LOD (mg/Kg)	LOQ (mg/Kg)
4-tert-OP	0.0090	0.03
4-n-OPEO1	0.0090	0.03
4-n-OP	0.015	0.05
4-n-NP	0.0060	0.02

3.2.4. Trueness

Trueness refers to the alignment between the obtained values' means and the enriched samples' known concentration.

This study quantified trueness as a relative bias percentage, the difference between the reference value and the average of the experimental results expressed as a percentage.

The results of the trueness study were evaluated based on the validation criteria of the matrix across four different concentration levels. The findings are presented in terms of bias (%) and recovery (%) (Table 3) [3]. The trueness of the method was confirmed by good recoveries of analytes, ranging from 105.0 to 99.5% (Table 3).

Table 3. Validation parameters for the four alkylphenols in fresh whole milk with a lipid percentage content of no less than 3% packaged in plastic bottles.

	Concentration (mg/Kg)	4- <i>tert</i> -OP	4-n-OPEO1	4-n-OP	4-n-NP
Trueness	0.1	−3.2	−4.4	−2.4	−2.0
(<i>n</i> = 3)	1.0	−6.6	−8.0	−5.0	−2.4
Relative bias (%)	2.0	−4.5	−3.6	−1.4	−3.3
Recovery (%)	0.1	96.8	95.6	97.6	98.0
	1.0	93.4	92.0	95.0	97.6
	2.0	95.5	96.4	98.6	96.7
Precision	0.1	2.6	2.3	2.5	2.1
(<i>n</i> = 3)	1.0	1.5	1.0	1.8	1.7
Repeatability (RSD%)	2.0	1.1	2.6	2.7	3.0
Precision	0.1	3.9	3.5	3.8	3.2
(<i>n</i> = 3)	1.0	2.3	1.5	2.7	2.6
Intermediate (RSD%)	2.0	1.7	3.8	4.0	4.5
Accuracy	0.1	−1.4; 5.0	−1.0; 7.8	−4.1; 4.9	−3.5; 3.6
(<i>n</i> = 3)	1.0	−3.0; 7.3	−0.9; 9.8	−3.7; 2.1	−4.5; 3.4
Tolerance limits β (%)	2.0	−3.6; 5.4	−3.3; 6.2	−2.9; 9.5	−3.2; 5.3
Uncertainty	0.1	6.8	5.6	7.2	8.8
(<i>n</i> = 3)	1.0	8.2	6.6	11.0	9.1
Relative expanded uncertainty (%)	2.0	9.7	5.5	6.5	7.4

3.2.5. Precision

Precision serves as an indicator of the method's relative error. It was assessed by examining the time-dependent repeatability (intra-day precision) and the intermediate precision (inter-day precision) across various concentration levels (0.10, 1.0, and 2.0 mg/Kg). The results were expressed as relative standard deviation (RSD%). The method's excellent reproducibility over short and extended periods was proven by RSD% values ranging from 0.25 to 4.5 (Table 3).

3.2.6. Accuracy

An accuracy profile graph represents the expected measurement tolerance intervals, visually enabling us to determine concentration levels that yield results within the acceptance limits and comprehend how effectively the procedure fulfills its purpose.

This validation approach reduces risks associated with future method applications. The procedure's total error (systematic and random errors) to be estimated is represented by the β -expectation Tolerance Interval (β -TI). Acceptability limits were set at $\pm 10\%$ (Figure 3).

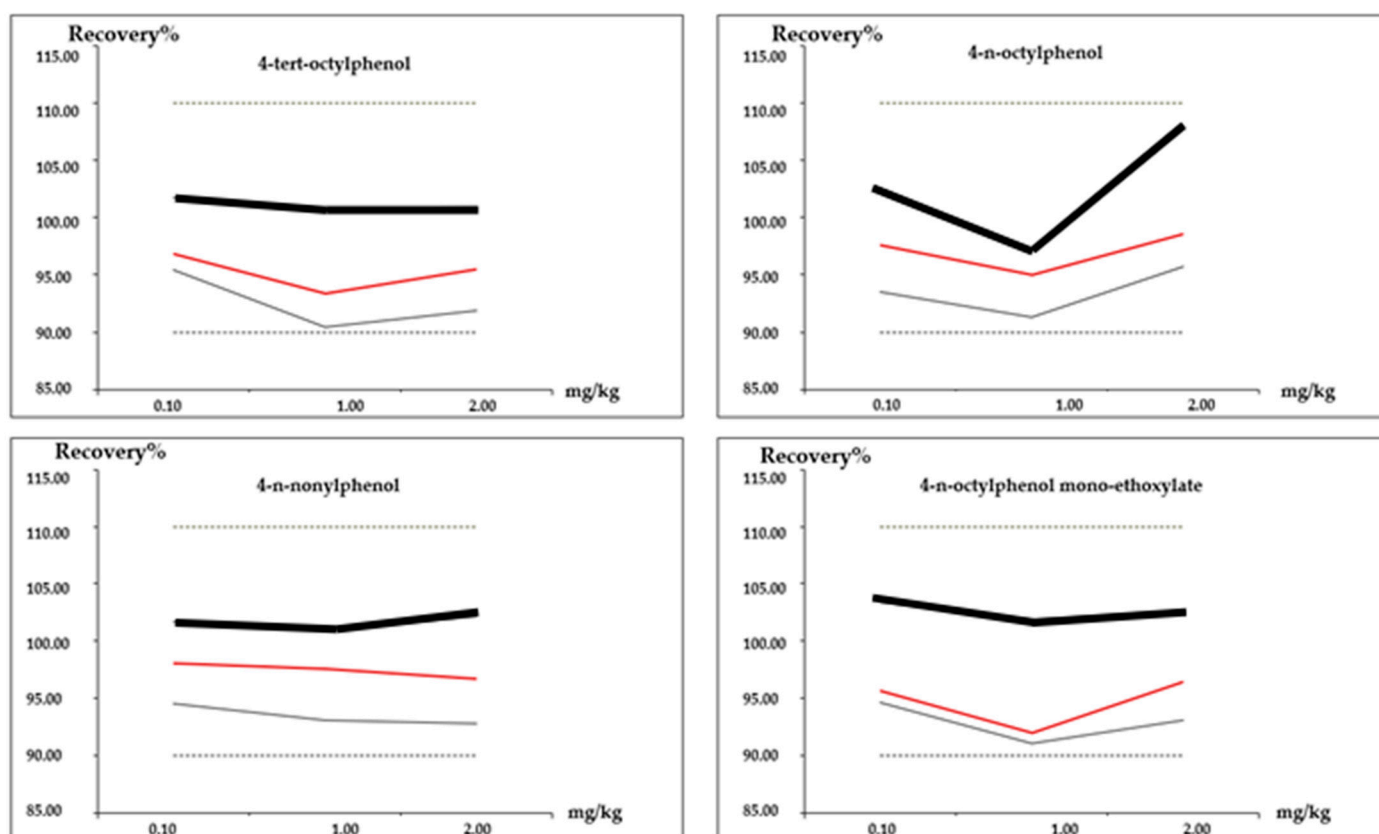


Figure 3. Accuracy profiles of the four extracted alkylphenols using the least-squares linear regression model. The dotted lines are the $\pm 10\%$ acceptance limits; the black lines are the upper and grey lines are the lower 95% expectation tolerance limits; the red lines are the recovery yield (%).

3.2.7. Uncertainty

The validation methodology for the accuracy profile enables the estimation of measurement uncertainty without any supplementary experiments [34].

Uncertainty denotes the range of values that may be reasonably assigned to the analyte. This study considered the extended uncertainty an interval within which the unknown “true” value can be observed with a 95% confidence level.

The relative expanded uncertainties (Table 3) were within a 5–11% range, signifying that the unknown true value is within a 5–11% range around the measured result, maintaining a confidence level of 95%.

4. Discussion

This study, addressing a critical need for reliable food safety analyses, develops and validates an innovative methodology for the precise dosage of APs in milk.

For the first time, SLE Chem Elut S cartridges, filled with a synthetic inert porous adsorbent, were used to eliminate molecules in milk that could interfere with AP dosing. The Chem Elut S cartridges give a rapid and user-friendly analysis, even for less experienced operators. They diminish matrix interferences, produce a high analyte concentration, and have a low environmental impact, necessitating minimal extraction solvents. Various solvents (ethanol, ethyl acetate, acetonitrile, and acetone) were evaluated for their use as mobile phase. The dichloromethane was chosen based on the AP recoveries. Matrix effects were rigorously assessed by comparing solvent standards and matrix-matched standards. The high extraction efficiency, significant recovery rates, and exceptional baseline stability, with no interfering peaks at the retention times of analyzed compounds in the HPLC-

DAD chromatogram, unequivocally validated the method's ability to reduce the impact of interferent molecules.

An HPLC-DAD apparatus was employed to quantify APs. An ODS Hypersil C-18 column was employed as a stationary phase to separate APs. The column choice was determined by prior research and the laboratory's resource availability [42–44]. The ODS Hypersil C-18 column gave rapid sample processing, efficient AP isolation, and short analytical run times.

The DAD detector, known for its ability to record UV spectra across multiple wavelengths, was chosen for its practicality and cost-effectiveness. While less sensitive than a mass spectrometer, the DAD is a widely accessible tool in laboratories and ideally suited for pollutant analysis [45].

The operational parameters of the HPLC system were meticulously optimized to ensure analytical performance (maximum sensitivity and precise measurements). An iso-cratric chromatographic run ensured rapid run time and an efficient and precise APs separation.

Finally, the test underwent rigorous validation following the strategy proposed by the Commission of the French Society of Pharmaceutical Sciences and Techniques (SF-STP) [33,34]. This strategy, known as “accuracy profiles”, redefines method validation by emphasizing the concept of “tolerance intervals”, which integrate the estimation of “total error” (a combination of systematic and random errors) and the measurement of “uncertainty” (the variability within the data dispersion).

This approach aligns validation with its primary goal: assessing “suitability for purpose” within pre-established “acceptability limit” criteria, defined as λ .

$$|Z - X| < \lambda$$

X = result;

Z = unknown “true” value.

The β -expectation Tolerance Interval (β -TI) represents the predicted range (with 95% uncertainty) within which upcoming measures are probable to fall.

This validation strategy allows decisions within clearly defined acceptability limits while effectively managing the risk associated with the method's use. It ensures a minimal probability of future assay results deviating by more than $\pm 10\%$ from the intended values with 95% confidence that the true values of measurements lie within the defined range.

5. Conclusions

This study developed and validated an innovative test to detect alkylphenols in milk. APs are harmful substances released by plastic packaging into milk that pose a significant human health risk, disturbing endocrine systems even at minimal concentrations. The suggested method employs the Chem Elut S cartridges (solid/liquid-phase extraction) for the first time to eliminate matrix effects and a chromatographic apparatus (reverse-phase HPLC-DAD) to quantify the APs. The method was validated using the accuracy profile approach, which considers bias and standard deviation to ensure reliability.

The reliability of the test was ensured by the absence of matrix molecules interfering with the analysis, a clear and consistent linear relationship between the independent and dependent variables, minimal discrepancies in both short- and long-term precision, and exceptionally low error margins in the dosage method.

It would be both strategic and beneficial to enhance public health and food safety for the research laboratories to be responsible for developing pre-validated tests for routine analyses since validation is resource-intensive and time-consuming. Ensuring ready acces-

sibility to reliable, accurate, and selective methods would significantly facilitate the work of commodity laboratories.

Future studies should focus on methods for reliable APs dosage across different food matrices.

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