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VALIDATION OF THE METHOD FOR DETERMINING POLYCYCLIC AROMATIC HYDROCARBONS IN PALM KERNELS FROM THE TONPKI REGION

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ABSTRACT

A rapid method for determining Benzo(a)Pyrene (B(a)P), Benzo(a)Anthracene (B(a)A), Benzo(b)Fluoranthene (B(b)F), Chrysene (CHR), Fluoranthene (FLA), Benzo(k)Fluoranthene (B(k)F), Dibenzo(a,h)Anthracene (D(a,h)A), Benzo(g,h,i)Perylene (B(g,h,i)P), and Indeno(1,2,3,c,d)Perylene (IdcP) in palm kernels using HPLC-UV has been validated. The validation procedure was conducted in accordance with the French standardization association method (NFV03-110, 1998), following the specific requirements of ISO/DIS/15753 applicable to High Performance Liquid Chromatography. The coefficient of determination values (R2) for the 9 studied PAHs ranges from 0.9915 to 0.9985, indicating linear calibration curves. Linearity testing demonstrates the normal distribution within the calibration range of 0 to 1000 µg/L. Most of the detection and quantification limits are below 0.2 µg/kg, confirming the high sensitivity of this PAH analysis method. The coefficients of variation obtained during repeatability tests (ranging from 0.89 for BaA to 2.28 for BkF) and reproducibility tests (ranging from 2.29 for BghiP to 4.88 for BaA) demonstrate the stability, reliability, and precision of the HPLC chromatograph. Therefore, the method for analyzing the 9 PAHs can be considered acceptable.

Keywords: polycyclic aromatic hydrocarbons (PAHs); palm kernels; Method validation; Analytical Chemistry; Environmental pollutants

INTRODUCTION

The almond of the oil palm fruit, also known as palm kernel and derived from the palm seed, is of crucial economic importance for Côte d'Ivoire. According to Yapi et al. (2020), Côte d'Ivoire is the second largest producer and exporter of palm kernel oil with an estimated annual production of 800,000 tons. A large part of the produced kernels are used by the population for their therapeutic, cosmetic, and nutritional benefits (Yapi et al., 2020). Industries are also interested in palm kernel oil due to its physicochemical and biochemical properties. For instance, palm kernel oil is used as a substitute for cocoa butter (Biswas et al., 2017). Nutritionally, palm kernel oil is rich in major nutrients (monounsaturated and polyunsaturated fatty acids), micronutrients (vitamins and trace elements), as well as secondary metabolites such as polyphenols and flavonoids. However, the presence of polycyclic aromatic hydrocarbons (PAHs) in palm kernels is a growing concern due to their toxicity and carcinogenic potential (Sparfel, 2018). Indeed, these molecules are included in the list of priority pollutants to be monitored in environmental samples according to the U.S. Environmental Protection Agency (US-EPA). Due to their physicochemical characteristics, PAHs are detected in ecosystems ranging from polar regions to tropical regions. They are found in the atmosphere, water, sediments, biota, and soils (Wilcke, 2007; Botta et al., 2014). Their lipophilic nature allows them to accumulate in the food chain (Shadi et al., 2012). Numerous studies have revealed the presence of PAHs in fish (Ake-Assi et al., 2012), meat and its derivatives (Rozentale et al., 2015), cola nuts (Shadi et al., 2012), cocoa beans (Sess-Tchotch et al., 2018), fruits (Paris, 2017), seafood, tea, rice, tomatoes, and potatoes (Bansal et al., 2015).

In order to regulate their content in food products, many organizations such as the European Commission have established regulations setting a maximum level of PAHs in food products (European Commission, 2011). The presence of PAHs at high levels (above international standards) in palm kernels produced in Côte d'Ivoire could constitute a significant health risk and even impact the economy by reducing access to international markets. Therefore, precise determination of PAH concentrations in palm kernels is essential to assess potential health risks and ensure compliance with regulatory food safety standards. This will involve validating the method of determining nine PAHs, namely Benzo(a)pyrene (B(a)P), Benzo(a)anthracene (B(a)A), Benzo(b)fluoranthene (B(b)F), Chrysene (CHR), Fluoranthene (FLA), Benzo(k)fluoranthene (B(k)F), Dibenzo(a,h) anthracene (D(a,h)A), Benzo(g,h,i)perylenes (B(g,h,i)P), and Indeno(1,2,3,c,d)perylenes (IdcP).

1. MATERIALS AND METHODS

1.2. Validation of the PAHs determination method

The validation of the method was carried out according to the method of the French Association of Standardization (NFV03-110, 1998) respecting the specific requirements of the standard ISO/ DIS/ 15753 applicable in High Performance Liquid Chromatography. The operating conditions for HPLC dosing of PAHs are presented in Table I.

Table I: Operating conditions for the determination of PAHs studied by HPLC

Pre column	Security guard, 20 mm x 4,6 mm
Column	Prevail C18, 150 mm x 4,6 mm
Detector	ultra-violet (UV), λ = 255 nm
Mobile phase	Acetonitrile/Water (50/100)
Injected volume	20 µL
Debit	2,5mL/minute
Column temperature	40°C
Rinsing solvent	Acetonitrile /Eau (50/50)
Duration of the analysis	6 minutes

1.2.1. Determination of linearity

The adequacy of the calibration curve to the linear model was verified by performing 5 replicates of a range of 6 concentrations (0µg/L, 100 µg/L, 250 µg/L, 500 µg/L, 750 µg/L, 1000 µg/L) for each of the 9 PAHs namely Benzo(a)Pyrene (B(a)P), Benzo(a)Anthracene, Benzo(b)Fluoranthene, Chrysene, Fluoranthene, Benzo(k)Fluoranthene, Dibenzo(a,h)Anthracene, Benzo(g,h,i)Perylene and Indeno(1,2,3,c,d)Perylene.

1.2.2. Determination of detection and quantification limits

Detection limits (LOD) and quantification limits (LOQ) were calculated from the analysis of 10 separate white matrix assays. These limits were calculated from the following formulae:

LD = Mx + 3S LQ = Mx + 10S
LOD: Detection limits,
LQ: Limit of quantification,
Mx: average on the 10 tests of blanks matrices,
S: standard deviation on the 10 blank matrix tests.

1.2.3. Repeatability and reproducibility tests

The repeatability test was performed by analysis of 10 reference sample tests. For the reproducibility test, 5 separate samples of reference samples were analysed at several days' intervals; a total of 15 tests. The

coefficient of variation was determined according to the formula below:

$$CV = \frac{S}{M_x} \times 100$$

S: Standard deviation on reference sample tests
Mx: Average on reference sample tests

1.4. Determination of the PAH content of palm kernel kernels

1.4.1. Extraction and concentration of PAHs from butterfat

The extraction of PAHs from palm kernel fat is performed using the method described by Sess-Tchotch et al. (2018). A test sample of 1g palm kernel oil was weighed in a tube to which was added 6 ml of potassium hydroxide in ethanol (KOH, 1N) and a magnetic bar (1). The assembly was then placed in an 80°C heating bath for 1 hour under agitation (450 revolutions/minutes) for saponification, then removed (2). A volume of 6 ml of cyclohexane was added to the tube which was again placed in a water bath at 80°C under the same stirring conditions (3). The tube was removed from the water bath after 5 minutes and then 4 ml of ultra-pure water (HPLC grade) was added (4). The whole was homogenized thanks to a vortex (Thermo SCIENTIFIC) for 1 minute at 1500 rpm/minute.

1.3. Method of determination of fat content

Fat extraction was determined using the method described in ISO 734: 2015. A test sample of 10 g of previously ground sample is weighed in an extraction cartridge. The fat is then extracted using a Soxhlet extraction device. The numerical expression of the extraction yield is given by the following formula:

$$Mg = \frac{m_1 - m_0}{m_e} \times 100$$

Mg: Fat content (%);
M1: Mass of flask containing concentrated fat (g);
m0: Empty balloon mass (g);

for 5 minutes at 3000 rpm (5). The contents of the tube were distinguished in two phases. Using a pastor pipette, the upper phase was removed and transferred to another tube (6). A volume of 3ml of cyclohexane was then added in the remaining phase (7) then steps (5) and (6) were resumed. The supernatant phase was then removed by heating to 40°C under a nitrogen stream (8). The resulting dry extract consisting of the PAHs was then recovered in 1 ml of acetonitrile, then homogenized at 1500 rpm for 1 minute.

1.4.2. Detection and quantification of PAHs by high performance liquid chromatography

For the HPLC analysis of PAHs, 20µl of the sample was injected into the mobile phase consisting of a mixture of acetonitrile and water (50/100, V/V). The mobile phase circulates at a flow rate of 2.5 ml/min in the stationary phase. PAHs were detected at a wavelength of 255 nm. A reactive white was injected at

the beginning of each series and a standard solution of known concentration was also injected after a series of 5 samples. Quantification of PAHs was performed from the absorption peak surface and PAHs content was determined in the samples from the calibration curves using the following formula proposed by Ehilé (2009):

$$C_i = \frac{A_i \times C \times V}{A_i \times m}$$

Statistical processing was performed using SPSS 12 and the statistical significance threshold was set at 0.05. Mean concentrations of Benzo(a)Pyrene (B(a)P), Benzo(a)Anthracene (B(a)A), Benzo(b)Fluoranthene (B(b)F), Chrysene (CHR), Fluorantheene (FLA), Benzo(k)Fluorantheene (B(k)F), Dibenzo(a,h)Anthracene (D(a,h)A), Benzo(g,h,i)Perylene (B(g,h,i)P) and Indene(1,2,3,c,d)Perylene (IdcP) were calculated with their standard deviation; then coefficients of variation were obtained to Ci: PAH content in sample (µg/kg); Ai: PAH peak area in sample solution; C: PAH concentration in standard solution (µg/L); V: final extract volume (mL); Air: PAH peak area in standard solution; m: Sample mass (g)

The PAHs content was reduced to the fresh sample mass according to the following formula:
$Cr = C \times Mg$

Cr: Actual PAH content (µg/kg); C: PAH
content obtained from palm kernel fat (µg/kg); Mg: Fat content of palm kernel kernels.

1.4.3. Determination of extraction yield Ten separate test samples from the

Ten separate test samples from the
reference sample were analysed to assess the recovery rate by the PAHs method.

$$\text{Recovery rate (\%)} = \frac{Cf}{C} \times 100$$

Cf: value assigned to the reference sample

C: measured concentration of the reference sample

1.5. Statistical analysis

express repeatability and reproducibility. The square of the Pearson correlation coefficient (R2) was calculated to assess linearity. The recovery percentage was calculated to express the extraction yield.

2. RESULTS

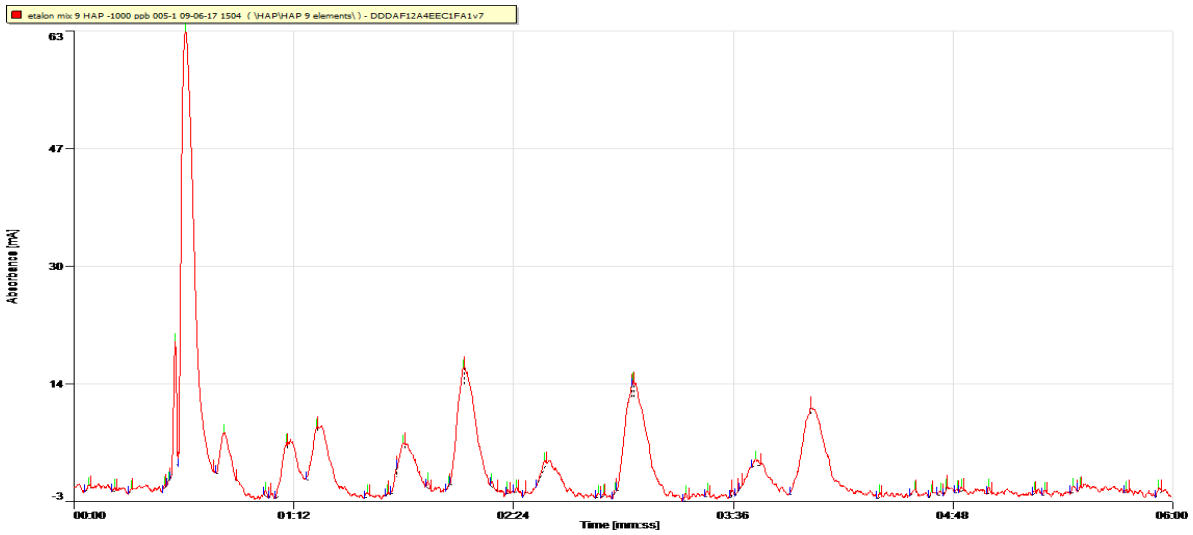
The validation of the method for determining BaP, BaA, BbF, CHR, FLA, BkF, DahA, BghiP and IdcP

focused on the tests of linearity, repeatability, reproducibility, accuracy, and detection and quantification limits. The results of this study are presented in Table II. Figure 1 shows the chromatogram of the test containing BaP, BaA, BbF, CHR, FLA, BkF, DahA, BghiP and IdcP at a concentration of 1mg/L. The square values of the Pearson coefficients (R2) of the 9 PAHs studied vary between 0.9915 and 0.9985. Limits of quantification are 0.09, 0.45, 0.17, 0.18, 0.88, 0.07, 0.04, 0.22, 0.13 µg/kg and detection limits are 0.04, 0.27, 0.07, 0.10, 0.55, 0.03, 0.02, 0.14, 0.09 µg/kg for BaP, BaA, BbF, CHR, FLA, BkF, DahA, BghiP and the IdcP. Coefficients of variation calculated for repeatability tests ranged from 0.89 for BaA to 2.28 for B(k)F. For reproducibility tests, coefisc of variation ranged from 2.29 for BghiP to 4.88 for BaA.

Table II: Validation data for the determination of PAHs content

Linearity

PAHs	Equation Calibration curve	CD(R^2)	CV repeatability (%)	CV reproducibility (%)	Extraction yield (%)	LD (µg/kg)	LQ (µg/kg)
B[a]P	y = 21294,6x	0,9954	2,24	3,82	97,45 ± 4,60	0,04	0,09
B[a]A	y = 19277,4x	0,9954	0,89	4,88	96,46 ± 4,77	0,27	0,45
B[b]F	y = 20948,1x	0,9929	2,08	3,89	102,73 ± 3,45	0,07	0,17
CHR	y = 13022,7x	0,9966	1,39	2,47	101,56 ± 3,84	0,1	0,18
FLA	y = 48258,3x	0,9985	0,99	3,82	100,41 ± 4,32	0,55	0,88
B[k]F	y = 9155,8x	0,9921	2,28	4,87	96,1 ± 3,5	0,03	0,07
D[ah]A	y =8018,72x	0,9944	1,88	2,64	97,92 ± 5,87	0,02	0,04
B[ghi]P	y = 20564,6x	0,9915	1,30	2,29	96,75 ± 5,51	0,14	0,22
IcdP	y = 9691,72x	0,9951	1,60	3,89	98,30 ± 5,2	0,09	0,13



Fluoranthene (1), Benzo(a)anthracene (2), Chrysene (3), Benzo(b)fluoranthene (4), Benzo(k)fluoranthene (5), Benzo(a)pyrene (6), Dibenzo(a,h)anthracene (7), Benzo(g,h,i)perylene (8) et Indeno(1,2,3,c,d)pyrene (9)

Figure 1: Chromatogram of 9 PAHs at 1 mg/L

3. DISCUSSION

The results of this work demonstrated the reliability of the method of determination of BaP, BaA, BbF, CHR, FLA, BkF, DahA, BghiP and IdcP in palmists in the Tonpki region. The square values of the Pearson coefficients (R2) are all approximately equal to 1. The resulting PAH calibration lines are therefore linear lines. The linearity test therefore highlights the normality of the distribution at the bounds of the calibration range of 0 to 1000 µg/L. As for the detection and quantification limits, they are mostly lower than 0,2 µg/kg, similar to those recommended by ISO/DIS-15753-2004. Thus, the method has a high sensitivity of the PAHs assay technique. The coefficients of variation obtained during the repeatability tests highlight the stability and fidelity of the HPLC chromatograph as well as its accuracy.

Reproducibility tests confirm the repeatability results and show the reliability of the extraction method of the nine PAHs. The results of the validation of the PAHs determination method are consistent with the recommendations of the experts (Zachara et al. 2017).

CONCLUSION

This study demonstrates the acceptability and reliability of the method for determiningBenzo(a)Pyrene, Benzo(a)Anthracene, Benzo(b)Fluoranthene, Chrysene, Fluoranthene, Benzo(k)Fluoranthene, Dibenzo(a,h)Anthracene, Benzo(g,h,i)Perylene and Indeno(1,2,3,c,d)Perylene in the palm trees. This research is of great importance for food security, public health and the agri-food industry in Côte d'Ivoire.

The results obtained will allow to assess the levels of PAHs contamination in palm trees and take appropriate measures to reduce the risks associated with their consumption and ensure their compliance with national and international standards.

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