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Optimization and Validation of a Modified Quick, Easy, Cheap, Effective, Rugged, and Safe Method Coupled with Gas Chromatography–Tandem Mass Spectrometry for the Determination of Eight Polycyclic Aromatic Hydrocarbons in Essential Oil-Rich Herbal Medicines

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Abstract

A modified Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) procedure coupled with gas chromatography–tandem mass spectrometry (GC–MS/MS) was optimized and validated for the simultaneous determination of eight polycyclic aromatic hydrocarbons (PAHs, including benzo[a]pyrene) in complex herbal matrices. This study aimed to address the severe lipophilic matrix interferences associated with conventional liquid–liquid extraction (LLE) in essential oil-rich herbal materials. A 2:3 (*v*/*v*) acetonitrile–acetone mixture was identified as the optimal extraction solvent, and C_{18} as the optimal clean-up sorbent. Validation using representative matrices (*Atractylodes macrocephala* and *Cinnamomum cassia*) met AOAC acceptance criteria for linearity, sensitivity, accuracy, and precision. Testing across 10 herbal medicines representing diverse plant parts showed acceptable recoveries and precision at low spiking concentrations, confirming broad applicability. Overall, this modified QuEChERS strategy provides a robust and efficient approach for routine monitoring of priority PAHs in complex botanical materials and supports future pharmacopoeial updates.

Keywords: benzo[a]pyrene; essential oil; gas chromatography–tandem mass spectrometry; herbal medicines; polycyclic aromatic hydrocarbons; Quick, Easy, Cheap, Effective, Rugged, and Safe

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1. Introduction

The Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method is an efficient sample preparation technique widely used to determine multiclass residues and contaminants in complex matrices. It is generally categorized into three approaches: the original unbuffered method, AOAC 2007.01 [1], and EN 15662 [2]. The original method uses simple, unbuffered extraction but may cause degradation or low recovery of pH-sensitive analytes [1,3–6]. Buffered methods, including AOAC 2007.01 and EN 15662, were developed to control pH during partitioning. AOAC 2007.01 uses an acetate buffer to improve compound stability and phase separation [7–10], whereas EN 15662 uses a citrate buffer to protect pH-sensitive compounds and is widely used as a regulatory method [11–14].

Following extraction, dispersive solid-phase extraction (d-SPE) removes matrix interferences using sorbents selected according to sample composition. Primary secondary amine (PSA) is widely used to remove polar interferences, including organic acids, sugars, and some fatty acids [15]. C_{18} (octadecylsilane) is added for lipid-rich matrices to remove nonpolar interferences [16,17]. Graphitized carbon black (GCB) removes chlorophyll and carotenoids from strongly pigmented matrices but requires careful optimization to avoid adsorption of planar analytes [18]. Z-Sep and enhanced matrix removal-lipid (EMR-Lipid) are advanced sorbents designed to selectively remove lipids from high-fat matrices while preserving hydrophobic analyte recovery [19,20]. Modified QuEChERS approaches are also used for limited sample amounts, complex matrices, or low analyte recovery, including μ-QuEChERS, which reduces solvent consumption, and customized solvent-to-salt ratios [21,22].

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants consisting of fused aromatic rings, primarily generated by incomplete combustion of organic matter from natural and anthropogenic sources [23]. The International Agency for Research on Cancer (IARC) categorizes PAHs into Groups 1, 2A, and 2B based on human carcinogenicity, with benzo[a]pyrene classified as a Group 1 carcinogen. Due to their carcinogenic and mutagenic properties, the United States Environmental Protection Agency (U.S. EPA) designated 16 priority PAHs, while the European Food Safety Authority (EFSA) established PAH4 and PAH8 as food safety indicators (Table 1) [24–26]. Regulatory authorities in the United States, Europe, and South Korea have established PAH residue standards for food products, with South Korea specifically setting maximum limits for BaP in target herbal medicines, including Rehmanniae Radix Prepata, Rehmanniae Radix, Mume Fructus, and Tsaoko Fructus [27–29]. The Korean Pharmacopoeia (KP) currently prescribes an LLE method for BaP analysis in herbal materials; however, for matrices particularly rich in lipophilic compounds, such as Mume Fructus and Tsaoko Fructus, an additional cleanup step using *N*,*N*-dimethylformamide (DMF) is required to minimize severe matrix interference [30]. Despite this modification, both conventional LLE and DMF-supplemented LLE remain suboptimal due to high organic solvent consumption and tedious, time-consuming sample preparation procedures (Figure 1). Consequently, there is an imperative need to develop a streamlined, efficient analytical method capable of effectively removing complex herbal matrices as well as high concentrations of lipophilic components (e.g., essential oils).

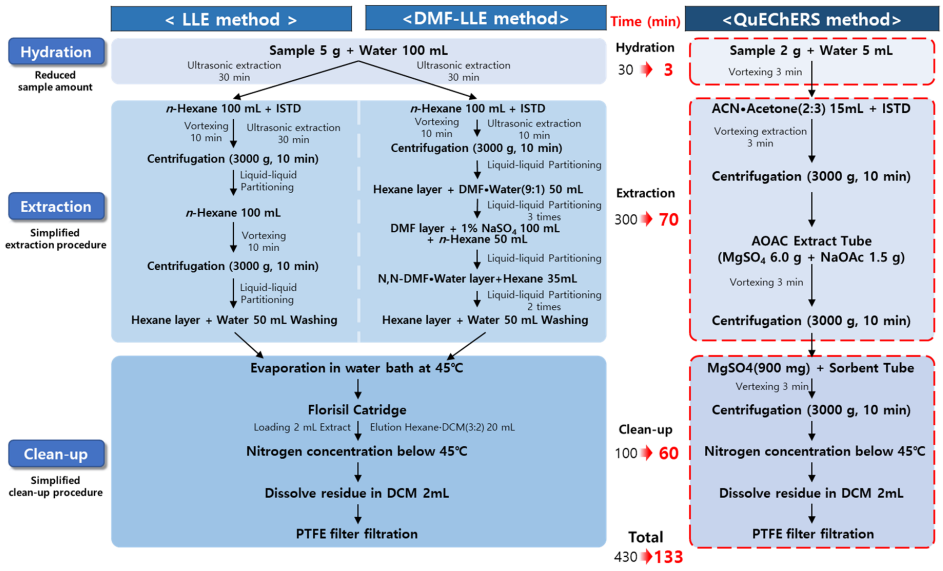


Figure 1. Comparison of sample preparation procedures and processing times between conventional Korean Pharmacopoeia (KP) methods (LLE and DMF–LLE) and the modified QuEChERS method.

Table 1. Physicochemical properties and international safety classification levels of 16 polycyclic aromatic hydrocarbons (PAHs).

PAHs *	Structure (# of Rings)	Molecular Weight (g/Mole)	Classification of IARC **	US EPA	EFSA	
				16 Priority PAHs	PAH8	PAH4
Naphthalene	2	128.17	2B	○		
Acenaphthene	3	154.21	3	○		
Acenaphthylene	3	152.2	3	○		
Anthracene	3	178.23	3	○		
Phenanthrene	3	178.23	3	○		
Fluorene	3	166.22	3	○		
Pyrene	4	202.26	3	○		
Fluoranthene	4	202.26	3	○		
Benzo[a]anthracene	4	228.29	2B	○	○	○
Chrysene	4	228.29	2B	○	○	○
Benzo[a]pyrene	5	252.32	1	○	○	○
Benzo[b]fluoranthene	5	252.32	2B	○	○	○
Benzo[k]fluoranthene	5	252.32	2B	○	○	
Dibenz[a,h]anthracene	6	278.35	2A	○	○	
Benzo[g,h,i]perylene	6	276.34	3	○	○	
Indeno[1,2,3-cd]pyrene	6	276.34	2B	○	○	

* Polycyclic aromatic hydrocarbons. ** International Agency for Research on Cancer.

To overcome the analytical constraints of conventional LLE, the present study applied the QuEChERS methodology for improved clean-up of complex herbal matrices, particularly targeting essential oil removal. In this modified QuEChERS approach, the extraction solvent was tailored to the physicochemical characteristics of eight PAHs, including BaP, and various sorbents, including C_{18}, PSA, GCB, Z-Sep, and EMR-Lipid, were systematically investigated to address diverse matrix interferences. Ultimately, this study aimed to optimize the modified QuEChERS conditions to mitigate matrix effects and ensure high recovery and sensitivity for the target PAHs in complex botanical materials.

2. Results & Discussion

The recovery rates of eight PAHs at varying extraction solvent mixing ratios are summarized in Table 2. When GCB was used as the adsorbent, none of the eight target PAHs were detected, indicating complete adsorption onto GCB. This is attributed to the planar geometry of PAHs, including benzo[a]pyrene, which has a strong affinity for the planar surface of GCB through π–π interactions, as previously reported [31].

To eliminate the influence of different adsorbents and evaluate the net effect of the extraction solvent ratio, the mean recovery rates across the four adsorbents (excluding GCB) were calculated and compared. Evaluation of these mean recovery rates showed that increasing the proportion of acetone enhanced the recovery of high-molecular-weight PAHs containing 5–6 fused rings (Table 2 and Figure 2). This trend is consistent with findings by Sun et al. [21], which demonstrated that extraction solvent polarity significantly influences the extraction efficiency of 5–6-ring PAHs. Conversely, when the MeCN-to-acetone ratio reached 1:4 (v/v), overall recovery declined across the target compounds. Therefore, based on these findings, an MeCN–acetone ratio of 2:3 (v/v) was established as the optimal extraction solvent ratio to ensure stable and high recovery values (%) for PAHs containing up to six rings.

Table 2. Recovery rates of eight PAHs under different extraction solvent ratios and sorbent types in modified QuEChERS optimization.

Solvent Ratio *	Sorbents	4 Rings				5 Rings			6 Rings
		BaA	Chry	BbF	BkF	BaP	IcdP	DahA	BghiP
5:0	C_{18}	94.92	101.29	101.54	93.33	89.94	87.56	86.07	80.75
	Z-Sep	95.98	89.69	84.70	78.80	91.68	73.73	77.99	64.55
	EMR lipid	96.86	116.10	109.26	84.47	113.39	108.61	110.05	102.77
	PSA	94.91	95.77	95.55	93.09	85.04	84.27	80.70	80.08
	GCB	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Average **	95.67	100.71	97.76	87.43	95.01	88.54	88.71	82.04
4:1	C_{18}	92.84	99.13	96.28	98.59	91.25	85.24	80.65	79.57
	Z-Sep	96.93	89.67	84.72	75.98	83.72	77.91	76.52	76.20
	EMR lipid	113.88	112.04	91.97	108.76	100.27	88.93	91.52	84.21
	PSA	90.44	98.33	92.24	89.51	90.68	85.60	86.25	77.69
	GCB	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Average	98.53	99.79	91.31	93.21	91.48	84.42	83.74	79.42
3:2	C_{18}	88.01	91.57	91.38	94.11	92.36	90.90	86.37	86.77
	Z-Sep	90.91	83.15	94.90	86.17	91.84	84.45	86.91	83.32
	EMR lipid	100.89	105.76	106.43	102.67	104.32	105.01	108.85	98.05
	PSA	88.20	92.44	93.80	92.04	93.91	96.63	92.29	89.84
	GCB	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Average	92.00	93.23	96.63	93.75	95.61	94.24	93.61	89.49
2:3	C_{18}	88.83	92.97	90.70	93.22	97.02	96.20	87.67	88.22
	Z-Sep	93.20	85.01	95.03	87.77	98.46	92.04	88.89	92.99
	EMR lipid	95.06	98.17	104.34	107.26	103.43	96.07	99.00	102.54
	PSA	92.29	92.43	93.66	92.60	97.05	102.27	100.97	101.73
	GCB	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Average	92.35	92.15	95.93	95.21	98.99	96.65	94.13	96.37
1:4	C_{18}	84.70	84.55	84.31	79.03	81.90	81.49	82.41	79.05
	Z-Sep	79.67	76.22	71.78	71.61	77.46	87.88	85.46	81.19
	EMR lipid	82.12	65.75	110.20	76.68	93.15	102.16	89.93	95.19
	PSA	97.92	86.42	81.33	77.96	88.92	91.75	89.27	93.45
	GCB	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Average	86.10	78.23	86.90	76.32	85.36	90.82	86.77	87.22

* MeCN–acetone (v/v). ** Average excluding GCB values.

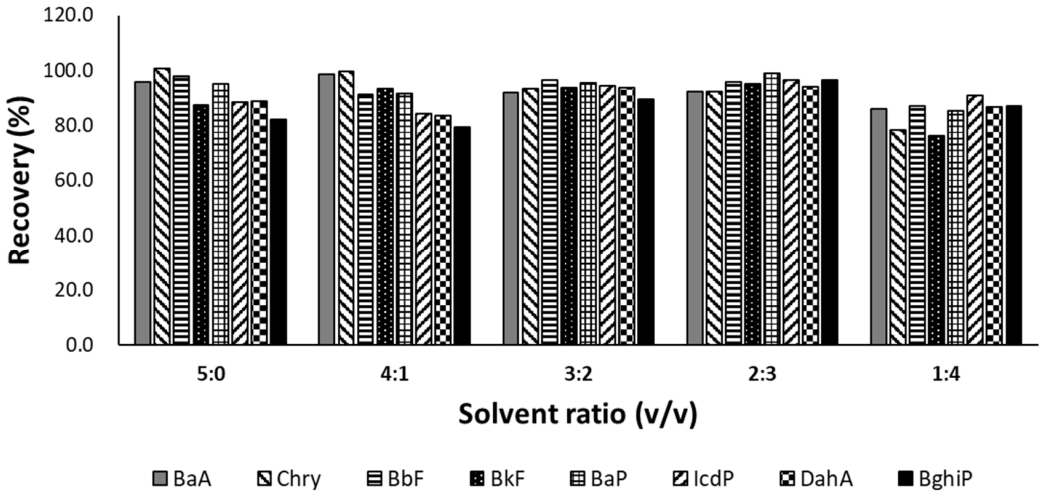


Figure 2. Average recovery rates of eight PAHs across different extraction solvent ratios using C_{18}, Z-Sep, EMR-Lipid, and PSA, excluding GCB.

At a solvent mixing ratio of 2:3 (v/v), all targeted PAHs showed high recovery values (%) ranging from 87.67% to 107.26% across the different sorbents (Table 2). However, significant differences were observed in analytical repeatability, expressed as relative standard deviation (RSD, %) (Figure 3A).

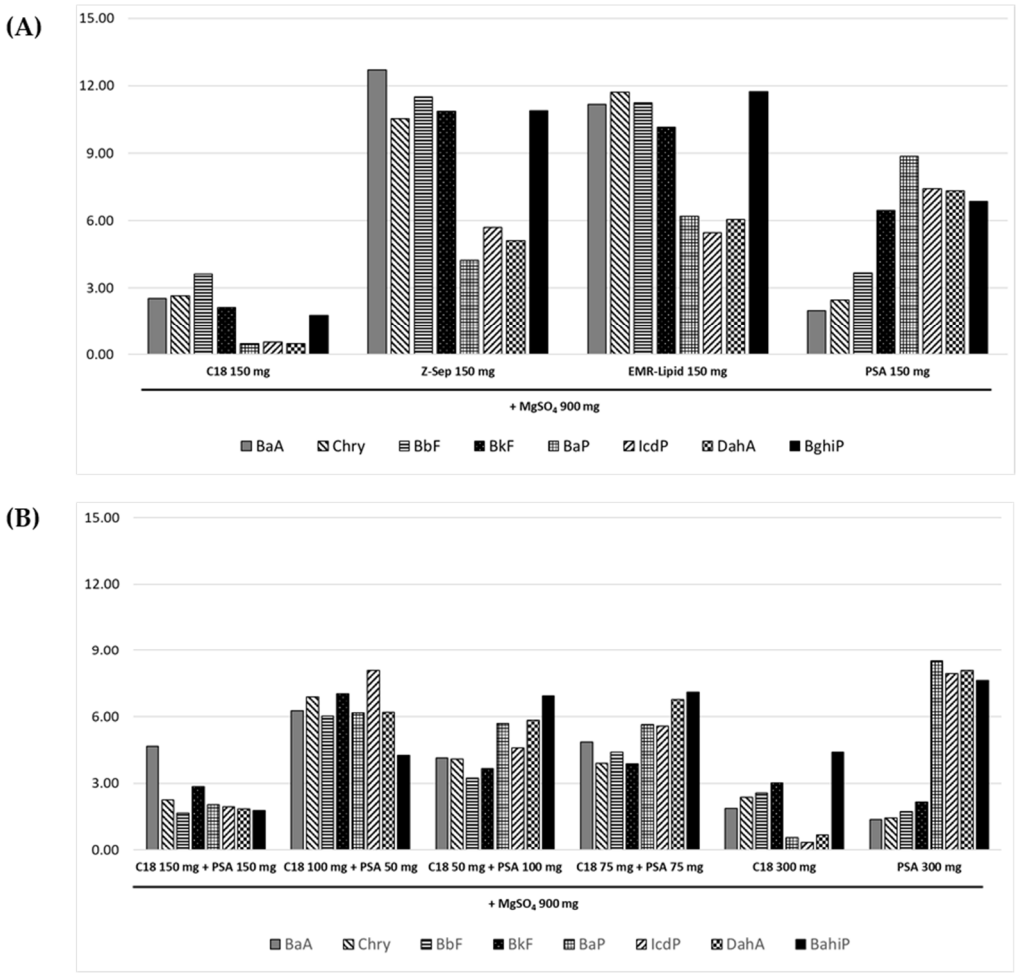


Figure 3. Comparison of relative standard deviation (% RSD) values for eight PAHs at a 2:3 solvent ratio using (**A**) single sorbents and (**B**) C_{18}/PSA combination treatments.

Among the four sorbents—C18, Z-Sep, EMR-Lipid, and PSA—evaluated across independent replicates ($n = 3$), C18, Z-Sep, and PSA satisfied the AOAC acceptance criteria for precision, showing descriptive RSD values below 15% for all target analytes. In contrast, EMR-Lipid exhibited RSD values exceeding the 15% threshold for 4-ring PAHs, which was presumably attributable to incomplete removal of co-extracted matrix interferences in the corresponding chromatographic retention window. In terms of descriptive comparison among the compliant sorbents, PSA yielded higher mean recoveries (92.3–102.3%), whereas C18 consistently showed the lowest RSD values across all eight target PAHs.

To explore whether combining sorbents could further improve performance, mixtures of C18 and PSA at various blending ratios were evaluated ($n = 3$, Figure 3B). Descriptively, the C18–PSA combinations did not yield higher recoveries than C18 alone and, for several analytes, resulted in higher RSD values. Therefore, based on the predefined quantitative selection criteria—(1) achieving acceptable recoveries (70–125%) and the lowest overall precision variability (RSD < 15%) across all eight PAHs, and (2) maintaining procedural simplicity without additional sorbent consumption—single-sorbent treatment with C18 was selected as the optimal clean-up condition.

The optimized QuEChERS condition (MeCN–acetone = 2:3, v/v; C_{18}) was validated using Atractylodes Rhizome and Cassia Bark. Validation parameters, including the LOD, LOQ, specificity, linearity, accuracy, and precision, were evaluated according to the AOAC guidelines. Because herbal medicines exhibit distinct physical characteristics depending on the plant parts used, applicability testing was subsequently conducted across herbal materials from various botanical parts.

Figure 4 shows the GC–MS/MS (QqQ) chromatograms and ion spectra of the eight target PAHs and three internal standards (Chry-d_{12}, BaP-d_{12}, and BghiP-d_{12}). Using multiple reaction monitoring mode, all 11 peaks were successfully separated without matrix interference. The calibration curves for the eight PAHs exhibited excellent linearity, with coefficients of determination (R^2) ranging from 0.9992 to 0.9999. The LOD and LOQ ranged from 0.16 to 0.30 ng/g and 1.00 ng/g, respectively (Table 3).

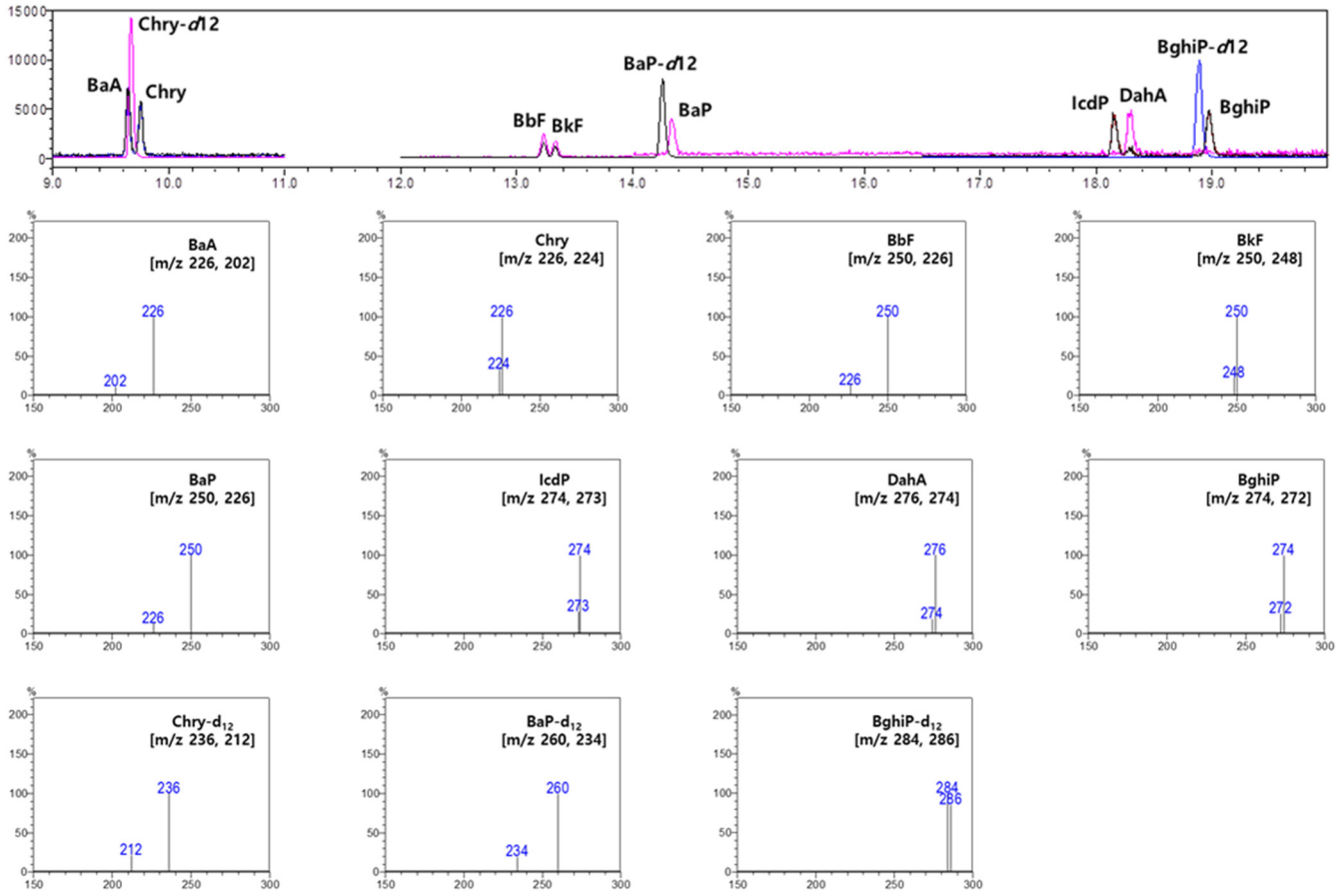


Figure 4. Total ion chromatograms and ion spectra of eight PAHs for specificity.

The quantitative reliability of the matrix-matched calibration models was further validated through residual analysis and back-calculation error evaluations. The residual plots for all eight PAHs showed a random distribution around zero, indicating no systematic heteroscedasticity across the calibration range. The back-calculated concentrations for each calibration standard level ($n = 6$) exhibited mean accuracies ranging from 82.6% to 104.7%, with relative errors (%REs) well within ±15% for higher calibration levels and ±20% at the lowest level, confirming the high robustness and accuracy of the calibration models (Table 3).

Table 3. Linearity, limits of detection/quantitation, and back-calculation accuracy of matrix-matched calibration curves (n = 6).

Analyte	Linear Range (ng/g)	Slope (a) *	Intercept (b) *	R^2	Theoretical LOD (ng/g)	Theoretical LOQ (ng/g)	Practical LOQ ** (ng/g)	Mean Accuracy Range *** (%)
BaA	1.0–20.0	0.0331 ± 0.0010	0.0047 ± 0.0016	0.9998	0.16	0.47	1.00	84.5–103.3
Chry	1.0–20.0	0.0212 ± 0.0002	−0.0008 ± 0.0019	0.9997	0.29	0.89	1.00	87.0–104.7
BbF	1.0–20.0	0.0344 ± 0.0010	−0.0024 ± 0.0029	0.9999	0.28	0.84	1.00	90.0–101.3
BkP	1.0–20.0	0.0455 ± 0.0021	−0.0009 ± 0.0034	0.9999	0.25	0.75	1.00	97.1–104.4
BaP	1.0–20.0	0.0259 ± 0.0008	0.0004 ± 0.0024	0.9999	0.30	0.91	1.00	88.0–102.5
IcdP	1.0–20.0	0.0448 ± 0.0009	−0.0094 ± 0.0039	0.9999	0.29	0.88	1.00	90.7–101.5
DahA	1.0–20.0	0.0391 ± 0.0011	−0.0115 ± 0.0019	0.9999	0.16	0.49	1.00	97.7–100.6
BghiP	1.0–20.0	0.0450 ± 0.0009	−0.0031 ± 0.0039	0.9992	0.29	0.87	1.00	82.6–102.7

* Slope (a) and intercept (b) are expressed as mean ± standard deviation from six replicate calibration curves ($n = 6$). ** Practical LOQ was conservatively set at the lowest calibration point (1.0 ng/g). *** Mean accuracy range derived from back-calculated concentrations across all calibration levels (1.0–20.0 ng/g).

Furthermore, the extent of matrix interference was quantitatively assessed by comparing the slopes of the matrix-matched calibration curves with those of the solvent-based calibration curves ($ME[\%] = [(Slope_{matrix}/Slope_{solvent}) - 1] \times 100$) (Table 4). The mean matrix effect (ME) values for the eight target PAHs in the representative herbal matrices ranged from −18.9% to −0.6% ($n = 6$). According to the SANTE guidelines [32], all ME values fell within the range of −20% to +20%, confirming that matrix effects were negligible and effectively minimized by the proposed clean-up protocol. Although the theoretically calculated LOQs ranged from 0.47 to 0.91 ng/g, a practical LOQ of 1.0 ng/g (the lowest calibration point) was conservatively adopted to ensure analytical precision and accuracy. At this practical LOQ level (1.0 ng/g), empirical verification using six replicate matrix-spiked samples ($n = 6$) yielded acceptable recoveries of 82.6–99.1% with relative standard deviations (%RSD) of 5.74–12.60%, fully satisfying the AOAC performance criteria.

Table 4. Quantitative assessment of matrix effects and empirical method validation at practical LOQ (1.0 ng/g).

Analyte	Solvent Slope (Mean ± SD)	Matrix Slope (Mean ± SD)	Matrix Effect (ME, %) *	Recovery at Practical LOQ (% ± SD)	Precision at Practical LOQ (% RSD)
BaA	0.0355 ± 0.0004	0.0331 ± 0.0010	−6.8	84.5 ± 9.0	10.68
Chry	0.0262 ± 0.0002	0.0212 ± 0.0002	−18.9	87.0 ± 5.3	6.12
BbF	0.0350 ± 0.0003	0.0344 ± 0.0010	−1.6	90.1 ± 9.8	10.83
BkP	0.0500 ± 0.0007	0.0455 ± 0.0021	−9.0	99.1 ± 6.8	6.90
BaP	0.0263 ± 0.0002	0.0259 ± 0.0008	−1.8	88.0 ± 5.1	5.74
IcdP	0.0450 ± 0.0004	0.0448 ± 0.0009	−0.6	90.7 ± 8.2	9.07
DahA	0.0423 ± 0.0004	0.0391 ± 0.0011	−7.5	98.1 ± 12.4	12.60
BghiP	0.0512 ± 0.0006	0.0450 ± 0.0009	−12.2	82.6 ± 6.6	8.03

* Matrix Effect (ME, %) = $[(Slope_{matrix}/Slope_{solvent}) - 1] \times 100$.

The modified method demonstrated excellent recovery across low, medium, and high fortification levels (2, 5, and 10 ng/g). Specifically, intra-day accuracy and precision were evaluated using three independently prepared replicate samples ($n = 3$) at each fortification level within a single day, whereas inter-day precision was assessed across three consecutive days using freshly prepared independent replicates each day ($n = 3$ per day, total $n = 9$). For Atractylodes Rhizome, intra-day recoveries, indicating accuracy, ranged from 74.7% to 106.8%, while inter-day (3 days) precision, expressed as RSD (%), ranged from 1.11% to 14.87% (Table 5). For Cassia Bark, intra-day recoveries ranged from 73.1%

to 111.5%, and inter-day precision ranged from 0.86% to 13.06% RSD (%) (Table 6). Thus, the validation results for both Atractylodes Rhizome and Cassia Bark satisfied the AOAC validation guidelines.

Table 5. Intra-day accuracy (recoveries) and inter-day precision (RSDs) for eight PAHs in Atractylodes Rhizome at three spiking concentrations (2, 5, and 10 ng/g).

PAHs	Intra-Day (n = 3)			Inter-Day (n = 3, 3 Days)		
	2 ng/g	5 ng/g	10 ng/g	2 ng/g	5 ng/g	10 ng/g
	Recovery (%)	Recovery (%)	Recovery (%)	RSD (%)	RSD (%)	RSD (%)
BaA	75.7 ± 3.6	74.7 ± 1.5	84.8 ± 1.4	5.14	8.20	1.11
Chry	106.3 ± 6.8	81.4 ± 7.4	81.5 ± 2.2	8.94	4.05	2.63
BbF	84.0 ± 4.5	82.5 ± 4.1	80.2 ± 5.4	11.29	3.02	1.69
BkF	96.0 ± 3.6	88.5 ± 5.8	81.6 ± 1.0	9.43	9.93	1.63
BaP	96.8 ± 8.7	79.5 ± 2.4	76.1 ± 2.4	5.17	3.54	2.01
IcdP	81.5 ± 6.1	90.6 ± 7.1	93.2 ± 1.9	14.87	1.81	1.43
DahA	106.8 ± 2.9	102.5 ± 10.3	101.4 ± 1.7	4.61	9.65	6.03
BghiP	84.5 ± 2.7	88.3 ± 4.8	80.3 ± 4.4	1.96	4.68	4.24

Table 6. Intra-day accuracy (recoveries) and inter-day precision (RSDs) for eight PAHs in Cassia Bark at three spiking concentrations (2, 5, and 10 ng/g).

PAHs	Intra-Day (n = 3)			Inter-Day (n = 3, 3 Days)		
	2 ng/g	5 ng/g	10 ng/g	2 ng/g	5 ng/g	10 ng/g
	Recovery (%)	Recovery (%)	Recovery (%)	RSD (%)	RSD (%)	RSD (%)
BaA	74.5 ± 0.2	76.9 ± 2.7	76.1 ± 2.3	9.66	9.93	4.81
Chry	103.0 ± 4.3	102.3 ± 4.1	96.5 ± 1.4	13.06	5.52	8.33
BbF	77.3 ± 2.3	75.7 ± 2.0	73.1 ± 1.4	1.70	10.41	0.86
BkF	94.3 ± 4.0	95.4 ± 1.2	82.2 ± 3.2	4.73	5.52	0.91
BaP	83.5 ± 1.4	77.4 ± 1.6	71.6 ± 1.3	6.11	3.40	1.16
IcdP	90.2 ± 3.5	88.8 ± 1.9	90.5 ± 1.4	8.01	2.39	4.05
DahA	111.1 ± 4.8	102.8 ± 2.5	108.6 ± 1.9	1.77	4.17	1.75
BghiP	89.3 ± 5.3	83.8 ± 2.6	78.9 ± 3.4	5.60	1.58	3.02

The results of method applicability testing for the ten herbal materials are shown in Table 7. At the low concentration level of 2 ng/g, recoveries and precision (RSD, %) ranged from 82.1 to 124.0% and 0.08 to 14.83%, respectively, falling within the AOAC acceptance criteria (70–125% for recovery and ≤15% for RSD). While these results suggest promising preliminary applicability at trace contamination levels across diverse medicinal plant parts, further multi-level fortification studies across the entire calibration range are warranted to fully account for matrix variations in essential oil and pigment compositions among different botanicals.

Table 7. Optimized QuEChERS method applicability results of the proposed method for 10 herbal medicines with different medicinal parts.

		A. lancea	*A. sieboldii*	*A. villosum*	*E. cardamomum*	*F. vulgare*	*I. verum*	*M. arvensis*	*M. fragrans*	*S. aromaticum*	*Z. schinifolium*
PAHs	Medicinal parts	Rhizome	Root	Fruit	Fruit	Fruit	Fruit	Aerial part	Seed	Flower	Pericarp
BaA	Recovery (%)	106.1 ± 10.2	107.9 ± 12.5	115.4 ± 5.7	113.0 ± 4.2	93.5 ± 1.6	118.3 ± 4.2	114.7 ± 6.1	104.7 ± 3.8	95.7 ± 12.3	112.9 ± 5.1
	RSD (%)	9.59	11.56	4.94	3.76	1.75	3.57	5.29	3.64	12.89	4.54
Chry	Recovery (%)	94.3 ± 2.1	85.2 ± 6.4	96.1 ± 2.3	115.1 ± 7.0	94.7 ± 2.8	102.3 ± 9.6	107.5 ± 3.0	103.3 ± 11.3	100.4 ± 9.1	110.8 ± 13.1
	RSD (%)	2.27	7.47	2.40	6.08	3.00	9.35	2.76	10.91	9.07	11.80
BbF	Recovery (%)	103.5 ± 7.2	100.8 ± 14.1	105.0 ± 3.6	95.8 ± 5.9	86.3 ± 3.1	113.4 ± 4.8	124.0 ± 0.8	99.3 ± 4.2	101.8 ± 1.8	106.8 ± 5.3
	RSD (%)	6.95	13.97	3.42	6.20	3.62	4.24	0.63	4.19	1.77	4.94
BkF	Recovery (%)	103.6 ± 13.6	109.6 ± 7.2	104.8 ± 0.6	107.7 ± 3.1	107.4 ± 5.3	108.4 ± 7.2	101.7 ± 5.3	87.0 ± 1.5	100.0 ± 2.3	97.0 ± 6.9
	RSD (%)	13.17	6.54	0.56	2.88	4.98	6.68	5.21	1.70	2.31	7.10
BaP	Recovery (%)	115.7 ± 3.7	94.3 ± 7.5	113.0 ± 2.5	87.4 ± 4.9	95.6 ± 9.6	105.2 ± 15.6	108.4 ± 2.7	112.3 ± 7.2	108.0 ± 6.9	97.1 ± 1.8
	RSD (%)	3.24	7.91	2.22	5.60	10.06	14.83	2.50	6.41	6.35	1.89
IcdP	Recovery (%)	91.1 ± 4.0	94.8 ± 9.3	104.8 ± 6.0	84.0 ± 7.0	109.8 ± 3.6	115.6 ± 4.4	116.9 ± 0.5	109.7 ± 6.7	108.2 ± 5.1	96.9 ± 1.8
	RSD (%)	4.38	9.82	5.68	8.35	3.24	3.85	0.47	6.10	4.72	1.82
DahA	Recovery (%)	93.1 ± 5.9	110.9 ± 3.7	109.2 ± 3.1	89.6 ± 8.5	111.9 ± 7.5	116.7 ± 6.1	111.5 ± 0.1	112.1 ± 3.7	108.1 ± 8.8	94.7 ± 1.1
	RSD (%)	6.30	3.32	2.86	9.51	6.74	5.27	0.08	3.27	8.15	1.16
BghiP	Recovery (%)	105.7 ± 11.0	108.6 ± 14.0	113.8 ± 4.4	82.1 ± 9.6	104.2 ± 7.6	110.3 ± 2.3	115.6 ± 7.6	111.3 ± 5.9	109.1 ± 8.9	96.9 ± 5.5
	RSD (%)	10.38	12.90	3.86	11.72	7.25	2.11	6.60	5.30	8.12	5.71

3. Materials and Methods

3.1. Chemicals and Reagents

Analytical standards of eight PAHs—Benzo[a]anthracene (BaA), Chrysene (Chry), Benzo[a]pyrene (BaP), Benzo[b]fluoranthene (BbF), Benzo[k]fluoranthene (BkF), Indeno[1,2,3-cd]pyrene (IcdP), Dibenzo[a,h]anthracene (DahA), and Benzo[g,h,i]perylene (BghiP)—and three internal standards (Chry-d_{12}, BaP-d_{12}, and BghiP-d_{12}) (Table 8) were of analytical grade (purity ≥ 99.0%) and purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (MeCN), acetone, dichloromethane (DCM), and distilled water (DW) were of LC grade and purchased from Merck (Darmstadt, Germany). Dispersive solid-phase extraction (d-SPE) sorbents—octadecyl (C_{18}), Z-Sep, GCB, primary–secondary amine (PSA), and enhanced matrix removal lipid (EMR-Lipid)—were obtained from Agilent Technologies (Santa Clara, CA, USA). The extraction salt mixture of anhydrous magnesium sulfate ($MgSO_4$) and sodium acetate (NaOAc) was supplied by Supelco (Bellefonte, PA, USA).

Table 8. Retention times of eight PAHs and three internal standards.

ISTD	STD	Retention Time (min)
Chry-d_{12} (9.44 min)	BaA	9.41
	Chry	9.52
BaP-d_{12} (13.97 min)	BbF	12.95
	BkF	13.05
	BaP	14.05
BghiP-d_{12} (18.57 min)	IcdP	17.83
	DahA	17.97
	BghiP	18.65

3.2. Preparation of Standard Solutions

Eight target PAHs and three internal standards were prepared as multicomponent mixed solutions. Individual stock solutions (100 µg/mL) were prepared in ethanol. A mixed working standard solution was prepared by diluting the eight stock solutions with DCM to 400 ng/mL for each analyte. Similarly, a mixed internal standard (ISTD) solution was prepared by diluting the three ISTD stocks with DCM to 1000 ng/mL for each compound. Both solutions were stored in amber glass vials at −20 °C in the dark to maintain stability and prevent photodegradation.

3.3. Samples

A total of 12 essential oil-rich herbal medicines were purchased from local markets in South Korea and authenticated by an expert according to Korean Pharmacopoeia (KP) standards. *Atractylodes macrocephala* (Atractylodes Rhizome) was selected as the representative matrix for modified QuEChERS optimization based on its regulated essential oil content in the KP and preliminary screening confirming no detectable PAH contamination. *A. macrocephala* and *Cinnamomum cassia* (Cassia Bark) were used for method validation. *C. cassia* was included as a challenging matrix because of its high essential oil content and potential for elevated PAH levels.

To assess applicability across medicinal parts, ten additional species with regulated EO standards in the KP were analyzed: *Syzygium aromaticum* (flower), *Myristica fragrans* (seed), *Illicium verum* (fruit), *Elettaria cardamomum* (fruit), *Amomum villosum* (fruit), *Asarum sieboldii* (root), *Zanthoxylum schinifolium* (pericarp), *Atractylodes lancea* (rhizome), *Foeniculum vulgare* (fruit), and *Mentha arvensis* (whole herb).

3.4. Sample Preparation and Extraction Method

A 2.0 g homogenized sample was weighed into a 50 mL centrifuge tube and wetted with 5 mL of DW for 3 min. After spiking with 10 µL of mixed standard (final concentrations of 2, 5, and 10 ng/g for method validation; 2 ng/g for method optimization and applicability testing) and 100 µL of ISTD solutions (final concentration of 50 ng/g), 15 mL of the optimized MeCN/acetone mixture (Section 3.6) was added. The sample was shaken for 3 min and centrifuged at 3000× *g* and 4 °C for 10 min. The supernatant was treated with 6 g of anhydrous $MgSO_4$ and 1.5 g of NaOAc, shaken for 3 min, and centrifuged again at 3000× *g* and 4 °C for 10 min. The organic layer was collected for clean-up.

3.5. Purification Method

The organic extract was transferred to a tube containing 900 mg of $MgSO_4$ and 150 mg of the sorbent mixture (Section 3.6), shaken for 3 min, and centrifuged at 3000× *g* and 4 °C for 10 min. The supernatant was evaporated to dryness under nitrogen, reconstituted in 2 mL of DCM, and filtered through a 0.2 µm PTFE membrane filter for GC–MS/MS analysis.

3.6. Optimization of the Experimental Conditions

To establish the parameters used in the aforementioned protocol, a systematic optimization was conducted.

Extraction solvent composition: The MeCN-to-acetone ratio was optimized using five binary mixtures at 1:0, 4:1, 3:2, 2:3, and 1:4 (MeCN, *v*/*v*). The mixtures were added to the wetted samples before salting-out to determine the optimal composition for PAH recovery.

Sorbent selection and mixing ratio: Clean-up efficiency was evaluated using five sorbents (C_{18}, PSA, GCB, Z-Sep, and EMR-Lipid). The most effective sorbents were then blended at various mass ratios to identify the optimal combination for removing essential oil interferences while maintaining PAH recoveries.

3.7. Instrumentation

A GC-QqQ-MS (GC–MS-TQ8040, Shimadzu) was used to analyze the 8 PAHs (Table 7) on a DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm; Agilent J&W Scientific, Folsom, CA, USA). The column temperature was initially 100 °C (0 min), increased to 230 °C at 55 °C/min, and then to 320 °C at 5 °C/min, with a 2 min hold. The total run time was approximately 22.36 min. Helium was used at 1.5 mL/min. The injector, interface, and ion source temperatures were 310 °C, 310 °C, and 250 °C, respectively. A 1 µL sample was injected in split mode (1/10) (Table 9). Quantification was performed using multiple reaction monitoring, with optimized transitions and collision energies listed in Table 10.

Table 9. GC–MS/MS instrumental conditions and parameters for the eight PAHs' analysis.

Instrument	GC-2030, GC–MS-TQ8040, Shimazu
Gas	He
Flow rate	1.5 mL/min
Column	DB-5MS (30 m × 0.25 mm, 0.25 µm)
Injection mode	Split mode (ratio, 10:1)
Injection volume	1 µL
Injection temperature	310 °C

Table 9. *Cont.*

Instrument	GC-2030, GC–MS-TQ8040, Shimazu		
Column oven temperature	Rate (°C/min)	Value (°C)	Hold Time (min)
		100	0
	55	230	0
	5	320	2
Interface temperature	310 °C		
Ion source temperature	250 °C		
Total running time	22.36 min		

Table 10. GC–MS/MS multiple reaction monitoring mode for the quantification and identification of eight PAHs.

Compounds	Precursor Ion	Product Ion		Collision Energy	
		Target Ion	Reference Ion	Target Ion	Reference Ion
BaA	228	226	202	18	27
Chry	228	226	224	33	45
BbF	252	250	226	39	27
BkF	252	250	248	24	57
BaP	252	250	226	39	24
IcdP	276	274	273	30	60
DahA	278	276	274	24	60
BghiP	276	274	272	27	60
Chry-d_{12}	240	236	212	36	24
BaP-d_{12}	264	260	234	27	36
BghiP-d_{12}	288	284	236	45	36

3.8. Method Validation

The QuEChERS-based method was validated for selectivity, specificity, linearity, LOD, LOQ, accuracy, and precision according to AOAC Official Methods of Analysis guidelines [33]. Method validation was evaluated using *A. macrocephala* and *C. cassia*. A three-level spiking experiment was performed at 2, 5, and 10 ng/g.

3.9. Selectivity & Linearity

Selectivity was evaluated by comparing blank matrix and matrix-matched standard chromatograms for interfering peaks at the retention times of the eight PAHs and three ISTDs. Linearity was assessed using matrix-matched calibration curves at 1–20 ng/mL across five concentration levels. The correlation coefficient (R^2) evaluated the goodness of fit.

3.10. Sensitivity

LOD and LOQ were determined from the SD of the intercept and mean slope of the calibration curve using the following equations:

$$LOD = 3.3 \times \frac{\sigma}{S}$$

$$LOQ = 10 \times \frac{\sigma}{S}$$

where σ is the SD of the y-intercept, and S is the mean slope of the calibration curve (n = 5).

3.11. Accuracy and Precision

Accuracy and precision were evaluated using recovery studies of spiked samples. Accuracy was expressed as percentage recovery, and precision as the relative standard deviation (RSD, %) of intra-day and inter-day (3 days) analyses.

3.12. Method Applicability

Method applicability was evaluated using ten herbal medicines with established essential oil content standards in the KP to assess performance across medicinal parts beyond the validation matrices (*A. macrocephala* and *C. cassia*). A single-level spiking experiment was performed at 2 ng/g. The selected species represented flower (clove, *S. aromaticum*), seed (nutmeg, *M. fragrans*), fruit (star anise, *I. verum*; cardamom, *E. cardamomum*; Amomum fruit, *A. villosum*; and fennel, *F. vulgare*), root (Asarum root, *A. sieboldii*), pericarp (Zanthoxylum fruit, *Z. schinifolium*), rhizome (Atractylodes rhizome, *A. lancea*), and aerial parts (Japanese mint, *M. arvensis*). All analyses were performed in triplicate, and suitability was assessed by recovery and precision (RSD, %) for each matrix.

4. Conclusions

In this study, an optimized QuEChERS-based GC–MS/MS method was developed and validated for determining eight target PAHs in complex, essential oil-rich herbal matrices. Systematic optimization identified an acetonitrile–acetone mixture (2:3, *v*/*v*) as the optimal extraction solvent, providing high recoveries of high-molecular-weight PAHs with up to six fused rings. Among the d-SPE sorbents, C_{18} effectively reduced matrix interferences without compromising reproducibility or causing analyte loss through planar adsorption, as observed with GCB. Validation according to AOAC and SANTE guidelines using Atractylodes Rhizome and Cassia Bark showed excellent linearity ($R^2 > 0.99$), negligible matrix effects (ME% within ±20%), sensitivity (LOD: 0.16–0.30 ng/g; LOQ: 1.00 ng/g), and accuracy and precision across low, medium, and high spiking levels (recoveries: 73.1–111.5%; RSDs < 15%). Furthermore, preliminary testing at a trace fortification level (2 ng/g) suggested promising applicability across ten diverse medicinal plant parts, including flowers, seeds, fruits, roots, pericarps, rhizomes, and aerial parts. Overall, this modified method demonstrated satisfactory analytical performance and high-throughput potential for the determination of eight PAHs in essential oil-rich botanical materials. While these results provide a foundational analytical basis that may inform future regulatory discussions and potential updates to the Korean Pharmacopoeia (KP), further validation of operational parameters—such as method robustness, carryover effects, and extract stability under minor variations in extraction conditions—is warranted before full implementation in routine quality control and official compendial monographs.

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Abbreviations

The following abbreviations are used in this manuscript:

DW	Distilled water
EFSA	European Food Safety Authority
IARC	International Agency for Research on Cancer
KP	Korean Pharmacopoeia
LOD	Limit of detection
LOQ	Limit of quantification
PAHs	Polycyclic aromatic hydrocarbons
RSD	Relative standard deviation
GCB	Graphitized carbon black

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