Advanced Analytical Techniques for Screening and Determination of Some Residues and Contaminants in Functional Foods

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List of abbreviations

ACN	Acetonitrile
AMR	Antimicrobial Resistance
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
APCI	Atmospheric Pressure Chemical Ionization
BLK	Blank
CAC	Codex Alimentarius Commission
CAP	Chloramphenicol
CCα	Decision Limit
CCβ	Detection Capability
CI	Chemical Ionization
CPS	Count Per Second
CV%	Coefficient of Variation Percent
DAD	Diode Array Detector
DLLME	Dispersive Liquid-Liquid Microextraction
d-SPE	Dispersive Solid Phase Extraction
DTT	Di-Thiothreitol
EA	Ethyl Acetate
EC	European Commission
EDTA	Ethylene Diamine Tetra Acetic Acid
EI	Electron Impact
ESI	Electro Spray Ionization
FAPAS	Food Analysis Performance Assessment Scheme
FDA	Food and Drug Administration
FOSHU	Japanese Foods for Specified Health Use
FQs	Fluoroquinolones
FSVs	Fat-Soluble Vitamins

GC	Gas Chromatography
GCB	Graphitized Carbon Black
CD-IMS	Corona Discharge Ion Mobility Spectrometer
GC-MS	Gas Chromatography Mass Spectrometry
GC-MS/MS	Gas Chromatography Tandem Mass Spectrometry
GPC	Gel Permeation Chromatography
HILIC	Hydrophilic Interaction Liquid Chromatography
HPLC	High Performance Liquid Chromatography
HR	High Resolution
HLB	Hydrophilic-Lipophilic-Balance
IAC	Immune Affinity Column
IDA	Information Dependent Acquisition
ID	Isotope Dilution
IF	Infant formulae
IL/IL	Ionic Liquid/Ionic Liquid
IQC	Internal Quality Control
ISTD	Injection Standard
LC	Liquid Chromatography
LCLs	Lowest Calibration Levels
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LLE	Liquid-Liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantitation
LPME	Liquid-Phase Microextraction
LSE	Liquid-Solid Extraction
M/Z	Mass/Charge ratio
MCQA	Matrix Clustering Quantification Approach
ME	Matrix Effect
MeOH	Methanol
MIPs	Molecularly Imprinted Polymers
MMC	Matrix Matched Calibration

MRL	Maximum Residue Limit
MRM	Multiple Reaction Monitoring
MRPL	Minimum Required Performance Limit
MSPD	Matrix Solid-Phase Dispersion
MU	Measurement Uncertainty
MWCNTs	Multi-Walled Carbon Nanotubes
NSC	Neat Standard Calibration
OCPs	Organochlorine Pesticides
OPPs	Organo-phosphorous Pesticides
PCA	Principal Component Analysis
PLE	Pressurized-Liquid Extraction
ppb	Part per Billion
PSA	Primary Secondary Amine
PT	Proficiency Test
PTFE	Polytetrafluoroethylene
QA/QC	Quality Assurance/Quality Control
QC	Quality Control
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
R-MMC	Representative Matrix-Matched Calibration
RPA	Reference Point of Action
RSD	Relative Standard Deviation
tR	Retention Time
SALLE	Salting-out Assisted Liquid-Liquid Extraction
SANTE	DG Health and Food Safety
SBSE	Stir Bar Sorptive Extraction
SFE	Supercritical-Fluid Extraction
SIM	Selected Ion Monitoring
SMPR	Standard Method Performance Requirement
SPE	Solid Phase Extraction
SPME	Solid-Phase Microextraction
SRM	Selected Reaction Monitoring

SRM	Standard Reference Material
TCs	Tetracyclines
TIC	Total Ion Current Chromatogram
TOC	Total Organic Carbon
TOF	Time of Flight
UA	Ultrasound-Assisted
VDIA	Variable Data Independent Acquisition
WHO	World Health Organization
WSV	Water-Soluble Vitamins

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Abstract

Functional foods provide nutritional and health benefits, yet they could be contaminated with residues like pesticides and polychlorobiphenyls. These residues affect the safety, quality, and consequently the commercial value of functional foods. Therefore, the validity and efficiency of residue determination methods constitute a major analytical concern. Reduction of matrix effect (ME) has always been the golden key for guaranteed sensitivity, selectivity, and high throughput analysis. This study aims for accurate determination and streamlined quantification of 200 pesticide residues in 16 matrices. Hence, a modified QuEChERS extraction protocol coupled to GC-MS/MS was then employed and separations were obtained in 25 min. Dilution of the final extracts of fresh and herbal samples was carried out to achieve an acceptable balance between sensitivity and peak characteristics. Dilution factors of 1x and 5x were selected for fresh and herbal samples, respectively. Principal component analysis (PCA) was then independently applied on the digitally exported total ion chromatograms (TICs) of the studied matrices and the calculated ME%. PCA score/loading plots of TICs demonstrated the key matrix constituents that influenced the obtained trends. Similarly, three main clusters were obtained after PCA of ME% indicating a dependent relationship between matrix type and the obtained effects. Out of the obtained three clusters, an appropriate representative matrix-matched calibration (R-MMC) was selected for ME compensation. Based on the EU validation guidelines, the proposed protocol was validated at 2 and 10 µg/Kg with acceptable method performance. Four proficiency testing (PT) and commercial samples were successfully analyzed. The proposed protocol would help laboratories to increase sample processing capacity and to ensure the safety of functional food products. This work should serve in setting standards that warrant the quality/safety of functional foods by national regulatory authorities.

On the other hand, Infant formulae (IF) are a significant category of special foods designed to replace a mother's breast milk. In this regard, IF quality and safety testing is critical to ensuring the health and development of this high-risk, vulnerable group. This study aims to develop and validate a streamlined assay protocol for water-soluble vitamins, vitamin D3, and some unauthorized antibacterial residues from various chemical classes. Hence, different IF compositions (cow and goat milk-based formulae) were extracted using an acetonitrile, water, and MeOH (3:4:3 v/v/v) mixture. Following that, the compounds were determined using a direct injection into an ultra-sensitive LC-MS/MS. Separations were carried out in a single chromatographic run over 12.0 minutes, using a Poroshell 120 EC-C18 column. Gradient elution was carried out with a buffered mobile phase of 10 mMole ammonium formate and methanol. The performance characteristics of the method were tested and validated in accordance with EU validation guidelines 808/2021/EC, 657/2002/EC, and Eurachem/CITAC. For all analytes, decision limits (CCα) and detection capabilities (CCβ), as well as LODs and LOQs, were well below the RPA of chloramphenicol (0.15 µg/kg) and the lowest achieved calibration levels (LCLs) per each studied compound. The LODs, LOQs, R^2, CCα, CCβ, percentage recovery, and CV% ranges were (0.0001-0.104 µg/mL, and 0.002-2.36 ng/mL), (0.00004-0.342 µg/mL, and 0.005-7.78 ng/mL), $\geq$ 0.990, (0.02-10.29 µg/Kg), (0.04-17.48 µg/Kg), (85.4-110.3%), and (0.8-19.3%), respectively. The reliability of the proposed assay was successfully attested through its application to thirty domestic commercials IF samples from global manufacturers as well as two previously analyzed PT samples. The results confirmed the practicality of the assay along with the suitability of the surveyed samples for infant consumption. This protocol would assist Egyptian regulatory authorities in making the proper decisions regarding the quality and safety of IF shipments while maintaining the least amount of disruption to the stream of commerce.

Keywords: Principal component analysis, GC-MS/MS, LC-MS/MS, functional foods, special foods, infant formula, pesticide residues, antibiotic residues, water-soluble vitamins, vitamin D3, matrix effects, representative matrix.