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Gas chromatography-mass spectrometric determination of organic acids by ion pair liquid extraction followed by in-situ butylation from aqua feed samples

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Abstract

A rapid and sensitive analytical method was developed to quantitatively determine organic acids (OAs) from fish feed samples extracted by ion-pair (IP) solvent extraction, followed by in-situ butylation and gas chromatography-mass spectrometric (GC-MS) analysis. The extraction of OAs was carried out with acetonitrile containing 10 mM tetrabutylammonium hydroxide (TBAH), and the analytes were derivatized to their butyl esters in the injection port of the GC-MS system. The developed method was validated in the range of 1–5000 ng/g, with recoveries ranging from 93–117%. The limit of detection (LOD) and limit of quantification (LOQ) of the method was 1–5 ng/g and 2–10 ng/g, respectively, yielding good linearity ($R^2 > 0.9990$) and precision with a relative standard deviation less than 10%. The proposed method was successfully applied to analyze OAs in sinking and floating fish feed samples. The analyzed samples showed the presence of benzoic, succinic, fumaric, glutaric, adipic, and phthalic acids in sinking feed samples; and benzoic, succinic, adipic, phthalic acids in floating feed samples, respectively.

Keywords

Activated charcoal extraction, fish feed, gas chromatography-mass spectrometry, ion pair liquid extraction, organic residue

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Introduction

Aquaculture is one of the fastest expanding industries around the world. Due to its high production and economic return potential, intensive fish production is increasing rapidly and becoming a vital enterprise worldwide whose revenue share increased from 7–39% due to the demand for cultured fish.[1] Infectious disease outbreaks have been described as a significant economic loss in aquafarming,[2] and to control them, various antibiotics are being used. The prolonged administration of low doses of sub-therapeutic levels of antibiotics as growth promoters (AGP)[3] and disease prevention agents may cause potentially harmful effects on human and animal health as well as the aquatic environment,[4] The European Union (European Parliament and Council Regulation (EC) No 1831/2003) has prohibited the use of AGP in feeds of food-producing animals from January 2006.[5] Researchers have proposed using organic acids (OAs), probiotics, prebiotics, and eubiotics as potential alternatives to antibiotic growth promoters in animal feed to maintain animal health and performance.[6] Among these, the usage of OAs (individual or blend mixture) as non-antibiotic compounds is an excellent alternative to antibiotics. It enhances the growth performance, which has been a route to continued global aquaculture industry expansion. Dietary OAs have been widely used around the world because of their antimicrobial activity, which can cause a pH decrease in the gastrointestinal tract (GIT),[7] acts against pathogenic bacteria, and eventually, improve nutrient utilization and animal growth performance.[8]

Many OAs used in the feed industry are generally recognized as safe (GRAS) antimicrobials.[9] The presence of OAs in piglets, poultry, and swine were reported

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previously.[10–12] The excessive use of OAs in aquafeed products makes the water acidic and leads to an increase in the acidity of the aquatic organisms, which may reduce overall productivity. Hence, the presence of OAs in the aquafeed samples has to be monitored with efficient and sensitive analytical methods. OAs such as benzoic acid, succinic acid, fumaric acid, adipic acid, and citric acid have often been used as food preservatives. The estimation of the OAs in food products is generally carried out with liquid chromatography-mass spectrometry (LC-MS) methods or by gas chromatography-mass spectrometric (GC-MS) methods after thorough sample preparation and followed by derivatization of the extracted analytes.

Several studies were previously reported to identify and quantify the OAs in various food and beverage samples[13] and environmental matrices.[14] Different analytical techniques including Ion exclusion chromatography (IEC),[15] Capillary electrophoresis (CE),[16] Nuclear magnetic resonance (NMR) spectroscopy,[17] High performance liquid chromatography (HPLC),[18] LC-MS,[19–21] Gas chromatography-mass spectrometry (GC-MS)[13,22] have been applied for the quantification of OAs from food products. Among all the techniques, the GC-MS technique has certain advantages such as low equipment cost, little solvent requirement, ease of analysis, and data reproducibility. However, the analysis of OAs by GC techniques needs a derivatization step such as silylation, methylation, or any other kind of alkylation processes that involve the use of toxic and explosive derivatization agents and include another step in sample preparation. The OAs are generally highly soluble in water, and hence their extraction from sample matrices is carried out with water by different extraction procedures such as Liquid-Liquid Extraction (LLE),[23,24] Solid-Liquid Extraction (SLE),[25,26] Ultrasound-Assisted Extraction (UAE),[27,28] Microwave-Assisted Extraction (MAE),[29,30] Accelerated Solvent Extraction (ASE),[31] Supercritical Fluid Extraction (SFE)[32,33] and Enzyme-Assisted Extraction (EAE),[34,35] and Solid-Phase Extraction (SPE).[36] The Extraction of OAs directly into the organic solvents leads to poor recovery of the analytes. The extracts need to be evaporated to dryness and reconstitution, followed by derivatization steps. The extraction of the acids from the matrices is affected by minerals and at high pH levels due to the formation of salts. Ion-pair (IP) solvent extraction is an attractive alternative extraction method by which the OAs can be selectively extracted with the aid of tetraalkylammonium halides or hydroxides. But the analytes must be subjected to derivatization steps even after the extraction with IP reagents for GC-MS analysis. The use of tetraalkylammonium hydroxides as IP agents was shown to be more efficient and lead to derivatization of the acidic analytes in the GC injection port. The methylation of the phosphonic acids when phenyl trimethylammonium hydroxide was used as IP reagent was reported previously.[37] It was also reported that the methylation did not occur when tetramethylammonium hydroxide was used for extraction, which means that the derivatization occurs only when there is sufficient hydrophobicity in the molecule. The use of tetrabutylammonium hydroxide (TBAH) as an ion-pairing reagent and its efficiency of butylation during GC-MS analysis of acidic degradation products of chemicals related to chemical weapons convention was reported.[38] The use of TBAH gave better peak shapes and high sensitivity when compared to the methyl derivatives. Hence, butylation is good alternative derivatization, and it reduces a couple of steps in the analytical procedure, improves peak shapes and detection of the analytes.

Hence, in the current work, the OAs in fish feed samples were extracted by IP solvent extraction with TBAH. The extracts were further cleaned up to remove hydrophobic lipids using activated charcoal (AC) and analyzed by GC-MS technique. The in-situ butylated OAs were quantified in selected ion monitoring (SIM) mode, and the method was validated thoroughly for all the required parameters. The validated method was applied to quantify OAs present in sinking and floating aqua feed samples.

Experimental

Materials and chemicals

The standards benzoic acid, succinic acid, fumaric acid, glutaric acid, adipic acid, pimelic acid, phthalic acid, and internal standard o-toluic acid (all the obtained chemicals are reference materials with purity >99%) were purchased from Sigma Aldrich (St. Louis, MO 63103, USA). HPLC-MS grade solvents such as acetonitrile, acetone, dichloromethane, ethyl acetate, and methanol were obtained from Biosolve (Dieuze, France). Multiwalled carbon nanotubes (MWCNTs), AC and TBAH 40% in water were procured from Sigma Aldrich (St. Louis, MO 63103, USA), DSC- 18 sorbent, QuE PSA/C18 cleanup cartridge was purchased from Supelco (Bellefonte, PA, USA). Nylon syringe filters with 0.22 μm particle size were purchased from Chromatopak (Mumbai, India). The ultrapure water was obtained from the Direct Q water purification system (E-Merck, Mumbai, India).

Preparation of standard solutions

The stock solutions of each of the organic acid standards, along with the internal standard were prepared at 1 mg/mL concentration in acetonitrile by dissolving 5 mg of each substance in 5 mL of acetonitrile (on calibrated Sartorius Model CPA225D balance with least count of 0.1 mg) and diluted to the required concentrations in a serial dilution process. All standards were stored at −20°C until analysis.

Sample collection

The commercial fish feed samples were procured from the local aquarium shops, and the real-time on-field fish feed samples were collected from the local fishery farms. The collected fish feed samples were finely homogenized using a mixer grinder and stored at room temperature until further analysis.

Extraction procedure

The finely powdered commercial fish feed samples (2 g), were first washed thoroughly three times with water to remove the dissolved OAs and the samples were vacuum dried and powdered using mortar and pestle and then fortified with the organic acid stock solutions at 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000 and 5000 ng/g concentration levels. The internal standard was added at a concentration of 200 ng/g. The fish feed samples were extracted by the solvent extraction process. The fortified fish feed samples were mixed with 2 mL of acetonitrile containing 10 mM TBAH and vortexed for 5 min. The sample was centrifuged at 16,000 x *g* for 5 min, and the supernatant acetonitrile fraction was separated. The extract was subjected to dispersive solid phase extraction cleanup with 3 mg/g of AC by vortexing for 1 min. The extract was again centrifuged at 16,000 x *g* for 5 min, and the supernatant solution was carefully decanted into another vial. The extract was filtered through 0.22 µm nylon syringe filters and evaporated to complete dryness by speed vacuum concentration. The residue was reconstituted in the 400 µL of acetonitrile and then subjected to GC-MS analysis.

GC-MS analysis

The GC-MS analysis of organic acid residues was carried out on the Agilent 7890B GC system coupled with Agilent 5977A mass selective detector and Agilent G4513A autosampler (Agilent Technologies, Palo Alto, CA, USA). The separation of the analytes was carried out on the HP-5 MS capillary column of the length of 30 m, 0.25 mm internal diameter and 0.25 µm film thickness (Agilent Technologies, Palo Alto, CA, USA). The injection port temperature was maintained at 300°C. The carrier gas Helium was used at a flow rate of 1.2 mL/min. The column oven was programed from an initial temperature of 80°C with an initial hold up time of 2 min, then ramped at 10°C/min to a final temperature of 230°C held for 3 min, and the total run time was 20 min. The samples were injected in split mode (10:1) at a flow rate of 1.2 mL/min. The GC-MS interface temperature was maintained at 280°C. The MS ionization was carried out in electron ionization (EI) mode with 70 eV electron energy. The source and quadrupole temperatures were maintained at 230°C and 150°C, respectively. The mass spectra were acquired in the full scan range from m/z 20–600 units. The quantification of analytes was carried out in SIM mode by selecting the major abundant peak as quantifier ion and the ion with the subsequent highest abundance as qualifier ion. The extracted ion chromatograms of the analyzed OAs are depicted in Figure 1. The molecular formulae, derivatized organic acid SIM ions, and retention times are represented in Table 1.

Results and discussion

Optimization of extraction method

Extraction solvent. The fish feed is a rich composition of proteins, lipids, vitamins, carbohydrates, sugars and minerals.[39,40] These samples are complex due to highly abundant lipids, proteins and other minerals. Hence, extraction with solvents by which the OAs can be extracted efficiently may also co-extract the majority of the lipids and proteins present in the samples. Therefore, to reduce the co-extraction of these unwanted interferences with organic acid residues, a suitable extraction solvent must be chosen for better recovery. The extraction performance of the organic acid residues from fish feed samples was investigated using hydrophilic solvents such as acetonitrile, methanol, and acetone; and hydrophobic solvents such as ethyl acetate and dichloromethane. Water was not chosen for extraction though most of the OAs are soluble in water due to its potential extraction capability of several proteins. The TBAH (1 M) stock solution was diluted to 0.1 M with methanol initially and then further diluted to 10 mM with the solvents. The fish feed samples were extracted as described in Extraction procedure, and the extracted residue samples were centrifuged at 16,000 x g, and the supernatant was separated. The extracts were further cleaned up with AC to remove soluble lipids and other hydrophobic impurities. It was observed that maximum extraction recoveries (98–117%) were observed in the acetonitrile extracts, followed by methanol, acetone, ethyl acetate and dichloromethane extracts. The lowest recovery was obtained in the dichloromethane extract due to its poor extraction capabilities of polar IP complexes. Hence, to ensure the maximum extraction of OAs, acetonitrile was selected as an extraction solvent. The extraction recoveries of the analytes with various solvents are depicted in Figure 2(a).

In-situ butylation of OAs with TBAH. OAs need derivatization due to their high polarities. The most frequently used derivatization method for the analysis of OAs are alkylation with diazomethane, esterification with alcohols in presence of mineral acids, and esterification with reagents like alkyl chloroformates. As these reactions need the use of hazardous substances, an in-situ derivatization method with TBAH was performed. The efficiency of TBAH for in-situ butylation in the injection port of the GC system was reported previously.[41,42] The OAs undergo a nucleophilic substitution reaction with TBAH by replacing the active hydrogen with an alkyl group at high temperatures. Hence the OAs are converted into butyl esters, and these butyl esters could be detected in the GC-MS system with good sensitivity. The following equation represents the derivatization reaction which converts the carboxylic acid to alkyl esters using TBAH.

$$[\mathrm{N}\,(\mathrm{CH_3})_4] + [\mathrm{OH}]^- + \mathrm{RCOOH} \rightarrow \mathrm{RCOOC_4H_9}$$

Optimization of AC cleanup. As fish feed is a complex matrix, it is vital to optimize the cleanup of the unwanted

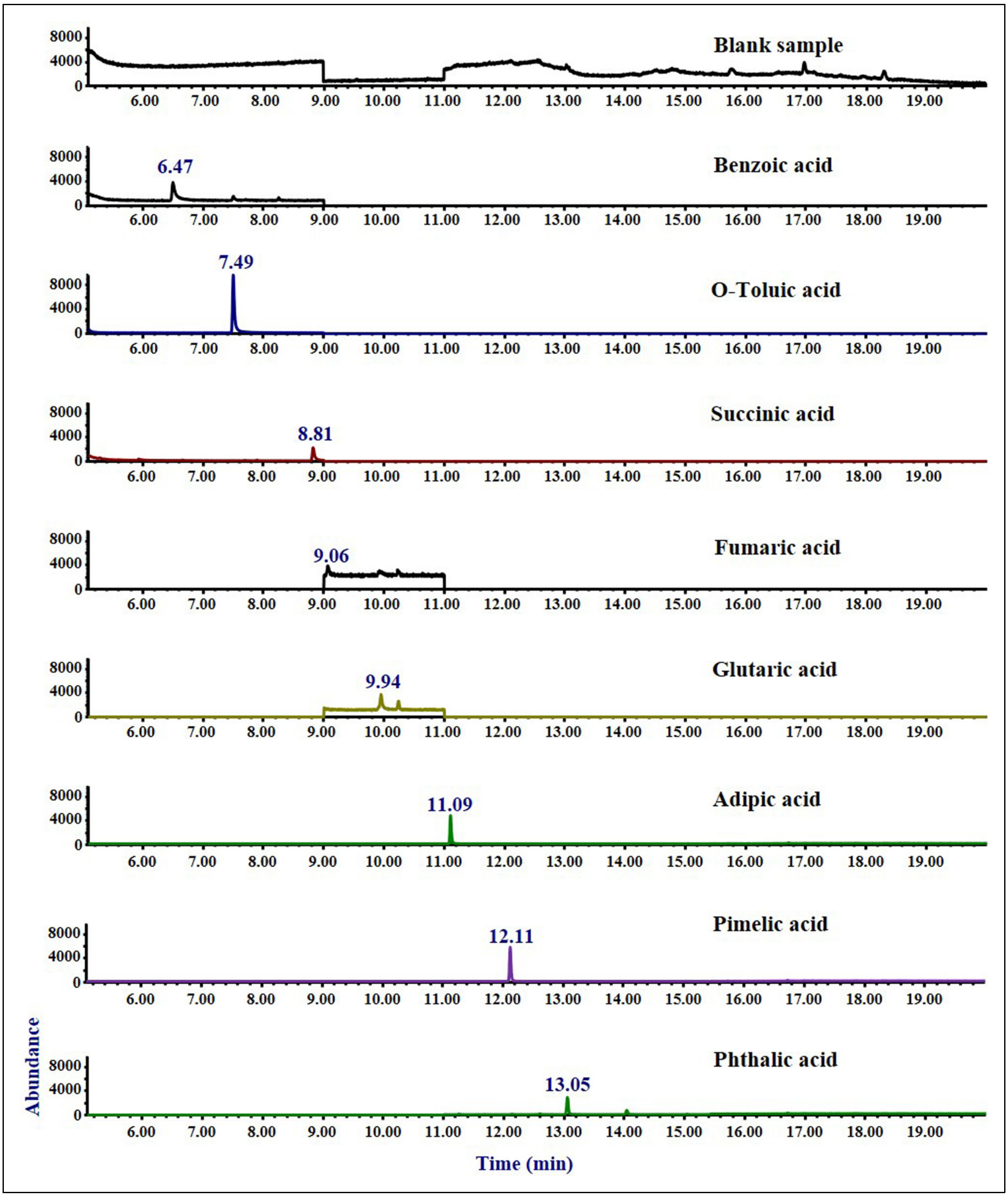

Figure 1. GC-MS extracted ion chromatograms of organic acids obtained from the fortified fish feed at LLOQ level.

residues in the extract to achieve better recoveries of targeted OAs. Hence, we have subjected the extracts to clean up with MWCNTs, AC, C18 sorbent, QuEChERS, and simple liquid-liquid techniques to cleanup the lipids without losing organic acid residues. The results showed that using AC, a considerable amount of background was reduced. The use of AC tends to extract hydrophobic constituents from the solvents; however, the extraction of hydrophilic constituents such as IP complexes from organic solvents is poor. Hence, the AC was selected for the removal of hydrophobic impurities. The recoveries were observed in the range of 85–117% measured at three different concentrations 10, 500 and 5000 ng/g, which were prepared in six replicates ($n = 6$). The recoveries in MWCNTs cleanup were less than AC, followed by C18 and QuEChERS. In general, activated carbon has a high surface area of up to 1200 m^2/g.[43] Whereas MWCNTs have similarities to AC in the carbon backbone bonding structure and the relative ease of chemical change. Due to the nanoscale and a large proportion of accessible micropores in their aggregated form, CNTs exhibit a high surface area than AC.[44,45] In this context, MWCNTs showed low recoveries in comparison with AC cleanup. The QuEChERS tubes contain primary

Table 1. Optimized GC-MS parameters for targeted organic acids.

S. no.	Organic acids	M. F[a]	M.W[b] (g/mol)	TBAH derivatization	SIM ions[c] Quantifier	Qualifier	R_t^d (min)
1.	Benzoic acid	$C_7H_6O_2$	122.12	178.0	105.0	123.0	6.47
2.	O-Toluic acid	$C_8H_8O_2$	136.15	192.0	118.0	91.0	7.49
3.	Succinic acid	$C_4H_6O_4$	118.09	230.0	101.0	157.0	8.81
4.	Fumaric acid	$C_4H_4O_4$	116.07	228.0	117.0	154.9	9.06
5.	Glutaric acid	$C_5H_8O_4$	132.11	244.0	115.0	171.0	9.94
6.	Adipic acid	$C_6H_{10}O_4$	146.14	258.0	184.9	129.0	11.09
7.	Pimelic acid	$C_7H_{12}O_4$	160.17	272.0	125.0	199.0	12.11
8.	Phthalic acid	$C_8H_6O_4$	166.14	278.0	148.9	104.0	13.05

[a]M.F–Molecular Formula.
[b]M.W–Molecular Weight.
[c]SIM–Selected Ion Monitoring.
[d]R_t–Retention time.

secondary amine, which has a strong tendency towards the OAs, and hence low recoveries were obtained in the QuEChERS cleanup procedure. Therefore, AC was selected to cleanup the extracts and remove the interferences during the extraction of OAs from the fish feed matrix. The recoveries obtained with various cleanup sorbents are represented in Figure 2(b).

Effect of amount of AC. AC is highly hydrophobic, has a high surface area, and can be used to effectively extract proteins[46] and lipids from the hydrophilic solvents.[47] The addition of AC needs to be optimized as it tends to co-extract the OAs and retain them. Hence, the amount of AC was optimized in the range of 1–5 mg/g. It was observed that a gradual increase in analyte recoveries to 117% till a concentration of 3 mg/g. Further addition of AC led to lower recoveries of analytes. Hence, the AC was added at a 3 mg/g concentration. Maximum recovery of the analytes was observed in the range of 95–117%. The extraction recoveries of the analytes with change in the amount of activated carbon are depicted in Figure 2(c).

Optimization of concentration of TBAH. The concentration of TBAH plays a vital role in the extraction of OAs and their derivatization. Increasing the concentration of TBAH leads to an increase in the peak broadening and area of tributylamine, which often interferes with the analytes. However, decreasing the ion-pairing reagent concentration results in very poor extraction recoveries of the targeted analytes. Hence, the concentration of TBAH was optimized in the range of 5 mM to 30 mM concentration. The recoveries were gradually increased up to 10 mM, and later on, significant low recoveries were found. Hence, we have used a 10 mM concentration of TBAH during the extraction process. The maximum recoveries of concentration of TBAH (88–117%) were depicted in Figure 3(a).

Method validation

The developed method was validated according to ICH Q2R(1) guidelines[48] for linearity, the limit of detection (LOD) and limit of quantification (LOQ), interday, and intraday reproducibilities of the method. The linearity studies were carried over a 1–5000 ng/g concentration range of the analytes. The obtained fish feed samples were washed thoroughly washed with water 3 times and analyzed by the developed method to check the presence of OAs. The sample that does not contain any OAs was selected as a blank sample and used to prepare matrix-matched calibration data. The chosen fish feed sample was fortified with the serially diluted stock solutions to 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000, and 5000 ng/g concentration levels and the organic acid residues were extracted as described in the experimental section (Extraction procedure). The prepared samples were subjected to GC-MS analysis. The results showed that the data obtained in the concentration range of 1–5000 ng/g was linear. The method has the LOD ranging from 1–5 ng/g observed at a signal to noise ratio of 3:1, and the LOQ was ranging from 2–10 ng/g observed at a signal to noise ratio of 10:1 respectively. The method was validated for intraday, and interday reproducibilities for six replicate samples at 10, 500 and 5000 ng/g concentration levels. The relative standard deviations were observed to be less than 10%. The validation parameters of the developed method are represented in Table 2.

Matrix effects and recovery

To estimate the matrix interference and percentage reduction in signal due to matrix effects, 2 g blank fish feed sample was extracted as described in Extraction procedure, and the standard solutions of organic acid residues at a concentration of 10, 500, and 5000 ng/g were added to the residue and reconstituted with 400 µL of acetonitrile and analyzed by GC-MS. The obtained data were compared to the standard solutions of similar concentrations prepared in the acetonitrile solvent. The percentage ratio of the decrease in signals was calculated. The obtained data indicated a $20 \pm 8.5\%$ reduction in the fortified matrix samples. The percentage recovery of the analytes was calculated by analyzing 2 g of blank fish feed samples fortified at 10, 500, and 5000 ng/g concentrations and the obtained data were compared to that of the

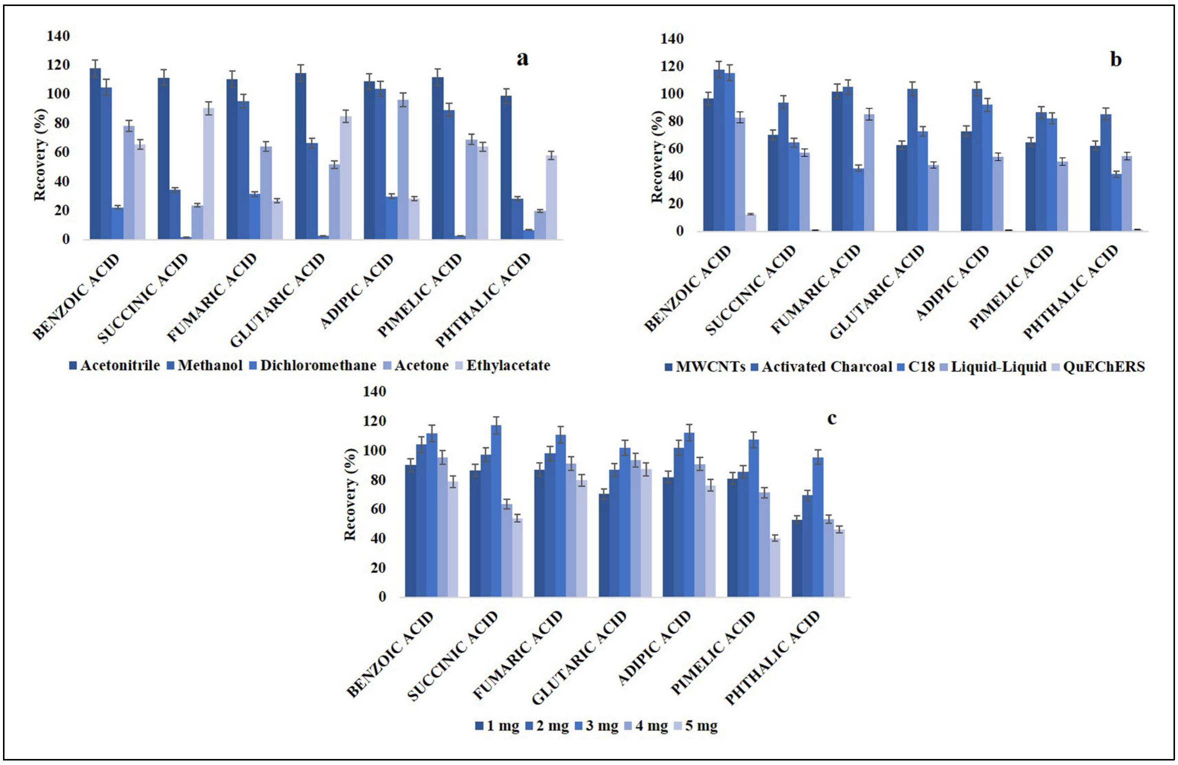

Figure 2. Recovery of the analytes at a concentration of 10 ng/g **a**, different extraction solvents; **b**, different cleanup methods; and **c**, amount of activated charcoal.

standard solution prepared in similar concentration levels.[49] The obtained data showed 93–117% recovery of the analytes and matrix effect between the range of −6 to 2%, which were found to be satisfactory, represented in Figure 3(b). The matrix effect and recoveries were estimated with the equations given below.

$$\text{Matrix Effect (\%)} = \left(\frac{A_a}{A_s}\right) \times 100$$

$$\text{Recovery (\%)} = \left(\frac{A_b}{A_a}\right) \times 100$$

where A_a = Peak area of the analyte of the sample spiked with the target OAs after extraction; A_s = Peak area of the analyte of standard solution; A_b = Peak area of the analyte of the sample spiked with the target OAs before extraction.

Carryover effects

The carryover studies were performed using a test sample fortified with OAs of high concentration (500 ng/g) in the fish feed matrix and a blank sample. After injecting a high concentration (n = 6) of OAs, the analysis was carried out to observe if there was any interference from OAs or a carryover of the previous sample in the blank. The blank sample injections were performed after the injection of high concentrations. The observed results showed that there was no carryover from the prior sample in the blank. The obtained total ion chromatograms of sample and blank samples are shown in the Supplemental Figure S1.

Stability of the OAs

The stability studies revealed that fortified fish feed samples with concentrations of 10, 500, and 5000 ng/g were stable when kept at room temperature and −20°C refrigeration temperature under short-term for 24 h and long-term for 72 h, respectively. Over the proposed hours, no significant change in the concentration of OAs was observed. The mean %RSD for samples stored at room temperature (25 ± 1°C) and refrigeration (−20 ± 1°C) was found to be less than 15%, indicating that OAs can be stored without degradation over the time intervals studied.

Determining the chemical stability of the OAs in the fish feed matrices was vital during method validation. Under various conditions, the stability of derivatized OAs in the autosampler was evaluated. After derivatization, the stability of the targeted OAs was tested under short term in the autosampler at room temperature for 8 h and in the long term for up to 24 h at concentrations of 10 ng/g, 500 ng/g, and 5000 ng/g of six replicates. These findings were compared to freshly processed fortified fish feed test samples of the same concentrations. OAs were found to be stable under all of the conditions mentioned. The results of the stability studies are represented in Table S1 of the Supplementary Material.

Robustness

The robustness studies were carried out by making small and deliberate changes to the validation parameters. The

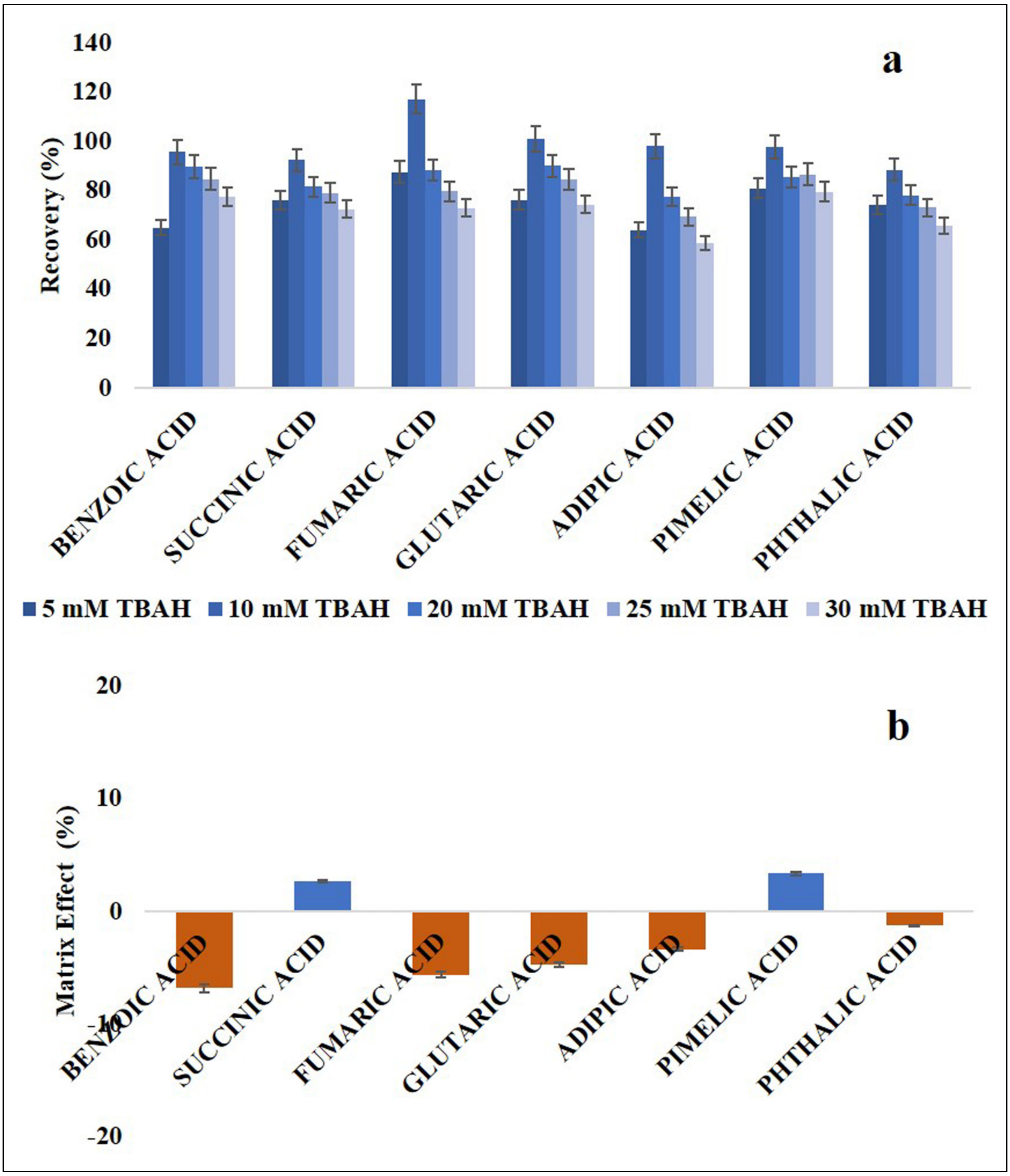

Figure 3. Recovery of the analytes at a concentration of 10 ng/g **a**, TBAH with different concentrations; and **b**, matrix effects.

effect of changes in carrier gas flow rate 1.0 mL/min (Flow minus), 1.2 mL/min (Control), 1.5 mL/min (Flow plus) and column oven temperature 200°C (Temperature minus), 230°C (Control), 250°C (Temperature plus) was investigated. The system appropriateness criterion is within the acceptance requirements in all permutations, indicating that the suggested technique is robust. The relative standard deviation of organic acid peaks derived from six replicate injections of the standard solution was observed to be below 15%, and the results are depicted in Table S2 of the Supplementary Material.

Analysis of OAs from fish feed samples

The performance of the validated method for simultaneous quantification of targeted OAs was evaluated by applying it to the sinking and floating fish feed samples. The results showed benzoic acid, succinic acid, fumaric acid, glutaric acid, adipic acid, and phthalic acid at a concentration of 2494.4, 81.1, 749.3, 78.6, 258.1, and 137.2 ng/g, respectively in 6 sinking feed samples; and benzoic acid, succinic acid, adipic acid and phthalic acid at a concentration of 263.8, 67.1, 670.8 and 873.5 ng/g, respectively in 4 floating feed samples. The

Table 2. GC-MS method validation parameters of organic acids obtained from the commercial fish feed.

						Validation accuracy (%RSD[d])		
S. no.	Organic acids	R^{2a}	Linearity Range (ng/g)	LOD[b] (ng/g)	LOQ[c] (ng/g)	10 ng/g	500 ng/g	5000 ng/g
1.	Benzoic acid	0.9995	1–5000	1	2	100.3 (8.2)	97.1 (4.9)	101.2 (1.9)
2.	Succinic acid	0.9994	1–5000	1	2	103.0 (9.3)	98.1 (7.9)	100.6 (6.5)
3.	Fumaric acid	0.9991	1–5000	1	2	96.5 (2.0)	104.5 (9.3)	103.7 (6.2)
4.	Glutaric acid	0.9997	1–5000	1	2	102.8 (7.6)	93.3 (6.4)	99.1 (8.8)
5.	Adipic acid	0.9990	1–5000	5	10	104.0 (8.8)	99.3 (9.5)	97.6 (8.4)
6.	Pimelic acid	0.9999	1–5000	5	10	117.0 (8.4)	98.9 (7.2)	99.3 (9.5)
7.	Phthalic acid	0.9993	1–5000	1	2	108.3 (7.6)	101.6 (9.5)	100.0 (6.2)

[a]Coefficient of Determination.
[b]LOD–Limit of Detection.
[c]LOQ–Limit of Quantification.
[d]The values in the parentheses represent %RSD valves (n = 6).

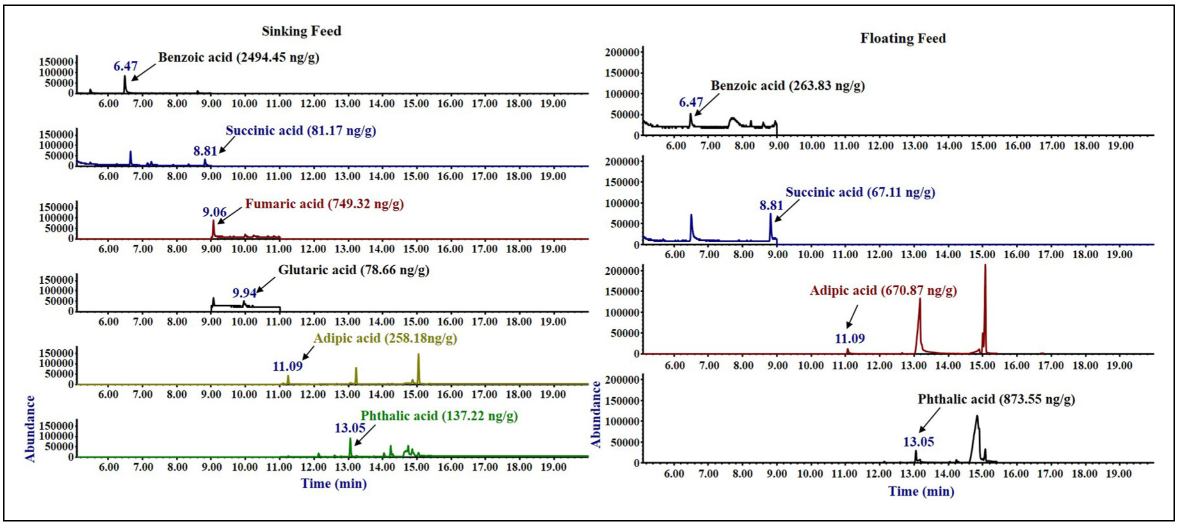

Figure 4. GC-MS extracted ion chromatograms of organic acids residues found in real fish feed samples.

GC-MS chromatograms of the fish feed samples in which the organic acid analytes were detected are presented in Figure 4. The results confirm that the found concentration of OAs residues is in the acceptable minimal levels, which were GRAS by the Food and Drug Administration-Code of Federal Rules (Title 21).[50] We have observed that benzoic acid is in the maximum concentration in sinking feeds. The proposed method is a simple and rapid technique that can be applied identification and quantification of the OAs in the aquafeed matrices.

Conclusions

A rapid, effective, and sensitive analytical method based on IP liquid extraction followed by cleanup with AC and GC-MS analysis was developed and validated for the quantitative determination of 7 organic acid residues from fish feed samples (sinking and floating). Significant reduction in the background and improvement in the recovery of analytes was observed when an optimized amount of AC was used for the cleanup of the extracts. The proposed GC-MS method showed good sensitivity with a LOD ranging up to 1–5 ng/g and the LOQ at 2–10 ng/g. The method showed good intraday and interday reproducibilities with satisfactory recovery and acceptable matrix effects. The developed technique was applied for the analysis of fish feed samples to quantify the organic acid residues. The results showed the presence of organic acid residues in the range of 67.1 to 2494.4 ng/g quantities. The proposed method can be used to evaluate the organic acid content in the medicated feed for routine analysis.

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Declaration of conflicting interests

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Supplemental material

Supplemental material for this article is available online.

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