Chapter

Current Trends in HPLC for Quality Control of Spices

Prafulla Kumar Sahu, Sukumar Purohit, Swarnajeet Tripathy, Durga Prasad Mishra and Biswajeet Acharya

Abstract

India, the land of spices and condiments, is endowed with a plethora of herbs, spices, and unusual plants. Spices have been used as flavoring and coloring agents in Indian society since time immemorial. Spices have also been shown to have antioxidant, antibacterial, anticancer, and anti-inflammatory properties. Assessing spices' taste, nutritional, and bioactive qualities during postharvest processing is critical for quality control and preventing adulteration. Various illegal colors are frequently used to adulterate spices for fraudulent trading operations. For instance, Sudan dyes are widely substituted with hot chili, red pepper, or tomato products; metanil yellow in turmeric; tartrazine, amaranth, and sunset yellow FCF in ginger and chili powder; and magenta III and rhodamine B in saffron. These adulterants degrade the flavoring, fragrance, cosmetics, medicinal, and preservative value of spices, their authentication is critical in quality control. Apart from these adulterants, various aflatoxins secreted after fungal contamination also cause quality degradation of spices. According to the literature evaluation, HPLC is a rapid and adaptable technique for efficiently identifying these compounds in spices. The proposed chapter summarizes application of HPLC for detection, quantification, and quality assessment of various spices. Some of the recently published work on the said topic from various search engines (Google scholar, Scopus, science direct, etc.) is mentioned in the chapter.

Keywords: analytical HPLC, spice and condiments, adulteration, aflatoxin, quality control

1. Introduction

Spice consumption has been a long-standing habit due to the great value of its color, flavor, pungency, and aroma properties. Spices are rich in lipids, proteins, minerals, and vitamins, in addition to their organoleptic qualities [1]. In addition, they are effective against microorganisms, oxidative stress, inflammation, diabetes, immunosuppressant, and mutation [2–5]. They are excellent for food preservation. As a result of its many advantageous effects, including the ability to purify blood and condition the skin, these spices have been mentioned in the ancient system of medicine such as Ayurveda, Unani, and Homeopathy. Besides the health benefits, global food habits include utilization of high spice levels, which makes the food more palatable and create an eye-catching garnish.

Global herb and spice market is valued at four billion USD and is believed to further grow up to 6–6.5 billion USD in next decade [6]. The increasing demand for spices is because of their flavor, aroma, taste, and color. Many of these spices and herbs include turmeric, ginger, garlic, chili, pepper, etc. used in every household worldwide. Apart from these whole spices, their powders (chili powder, turmeric powder, ginger powder, pepper powder, etc.) are also used for seasoning. Unfortunately, these powders are often contaminated with chalk powder, dyes, and many other chemicals to enhance the bulk and colored texture of the spice [7]. This food fraud practice can be detrimental to health condition of consumers. For instance, various reports suggest that nut protein mixed with cumin can cause anaphylaxis [8]. Yellow chalk powder is often mixed with turmeric powder, which may cause severe nausea, vomiting, and loss of appetite to the consumers [9]. Mixing olive leaves with oregano is another example of indirect type of food fraud, which can cost toxicity and mutagenicity to the customer [10].

Intentional addition or substitution of a substance with a structurally similar substance to enhance its quantity and decrease its production and processing cost for economic gain is called economically motivated adulteration [11]. This practice encourages fraudsters to mix various harmful illegal dyes with spices to make them more appealing to the customer. For instance, Sudan one and Sudan four are mixed with turmeric, chili, curry, pepper, etc. [12]. Rhodamine B is mixed with paprika, sumac, chili, etc. [13]. Para red is an illegal dye often mixed with cayenne pepper, chili, and paprika to enhance the color of the spice [13]. These above-mentioned banned dyes are genotoxic and carcinogenic in nature. Therefore, identifying these adulterants from herbs and spices has become an important step for their quality control.

Owing to the huge economic potential of the spice market, herbs and spices are heavily adulterated. Very often they are adulterated with low-quality and substandard products. This practice further enhances the possibilities of contamination. Improper storage and handling have a significant role in quality degradation of spice, which includes microbial growth [14]. Besides bacterial contamination, spices are more susceptible to fungal contamination. Mycotoxins are toxic materials released to the fungus-contaminated spices and herbs. Aflatoxins are a type of mycotoxins that are produced by certain fungi causing severe contamination in many agricultural crops including spices [15]. Aflatoxin contamination in spices occurs during their harvesting, drying, and storage [16]. These toxins must be identified and approaches should be considered to minimize such contamination for the quality enhancement of spices.

To verify the authenticity of herbs and spices due to the surging trends in spice contamination, many analytical techniques are taken into consideration. Most relevant techniques include spectroscopy and chromatography [17]. Visual inspection and microscopic methods are also considered, but they require huge manpower, trained professionals, and more analysis time. Many DNA-based methods, such as random amplified polymorphic DNA (RAPD), are also used for the detection of adulteration in spices. However, the authenticity of the result reproducibility is a matter of discussion [18]. High-performance liquid chromatography (HPLC) is a rapid, reliable, accurate, sensitive, and highly specific technique considered the gold standard for the detection of adulterants [19]. This chapter gives a complete overview of the ancient use of spices in India and their trading and fraudulent activities followed over the years to adulterate spices. Further, the report summarizes various recent findings based on identification of different adulterants including synthetic dyes, herbicides and pesticides, starch and fillers, etc. through HPLC methods that are mentioned. In addition to this, role of HPLC in quality assessment of drugs bearing essential oil and mycotoxins has been discussed elaborately. A comparison study between HPLC

analysis and other spectroscopic and chromatographic techniques is also discussed in this manuscript. This chapter will be the first documentation of its kind to cover the role of HPLC in quality boosting of different herbs and spices.

1.1 History, trivia, and background about spices of India

Spices have various purposes, including cooking, aroma, personal care, medicine, and long-term food preservation. Most countries that import goods find spices important. There are many aromatic spices and flavors in Indian history. Spices have been pivotal in many realms, from mythology and the Middle Ages to modern politics and economics. Spices were used as exchange and gifts for marriages, war treaties, political tradings, etc. [20–22].

Spices have long been a globally traded commodity. Spices were one of the most important components of trade from the Indian subcontinent to the Roman Empire throughout the first to third centuries CE [23]. The Middle East initially used spices circa 5000 BC, which further moved through Egyptians exchanged between 3000 to 200 BC. They were cultivating garlic to use as a fragrance in the process of mummification. The Romans dominated the trade of spices including nutmeg, cinnamon, pepper, cloves, and ginger from 200 BC until 1200 AD. According to Indian history, in 1497, Portuguese explorer Vasco de Gama lured princes of India with spices such as ginger, pepper, and cinnamon. In 1663 AD, the Dutch people acquired exclusive permission for pepper trading with India and controlled Asian spices by the 17th century. In the late 17th century, the French became a superpower and stole cloves, cinnamon, and nutmeg from the Dutch. In 1780–1799, British took seized spice trading centers. By the year 1672 America joined the spice race through its geopolitical and economic eminence.

India is the world's greatest spice grower, accounting for 75% of worldwide spice production. The overseas trading of spices by India in 2018–2019 was 0.85 million tonnes ($2.25 billion). The spice market's growth in recent years is a testament to the popularity of Indian spices abroad. Flavor, color, aroma, preservation, and therapeutic characteristics give spices considerable economic worth. Important seasonings, tree spices, seed spices, and miscellaneous spices all make up the spice family [24]. It is estimated that between 6.8 and 12.5 percent of spices are lost after harvest [25]. Spice businesses also experience pollution and adulteration. Adulteration causes health dangers, dangerous products, and poor quality.

The spice sector is constantly challenged by adulteration, which can take the form of introducing low-grade, harmful, or low-quality commodities as well as extraneous chemicals. Adulteration not only reduces the overall quality and authenticity of a product but it can also have a negative impact on consumer health, including the development of long-term conditions such as paralysis, cancer, and a compromised immune system because of the presence of poisonous products inside the human body [26]. Spice adulteration business stands at around $30–$40 billion per year as per the estimate given by Global Food Safety Forum. Adulteration is done to profit from cheaper raw materials and meets overpopulation's food need. Lack of knowledge about adulterants in spices lowers their marketability and safety.

1.2 Importance of spices for the Indian economy

Spices are popular everywhere. India is the largest exporter and producer of herbs and spices worldwide. Spices are a highly traded commodity. Spice imports from developing countries like India dominate the worldwide market [27].

In India, the best quality spices are cultivated in the western ghat, Coorg, and Malabar region of southern states. Apart from that, other Indian states such as Madhya Pradesh, Kashmir, and Uttar Pradesh depend on their geo-climatic circumstances. In Uttar Pradesh, cumin, coriander, fennel, and black seed are commercially grown [28]. Spices are an ideal crop for small-scale farming in India. This firm may provide the family with more career options and emergency cash. Spices are a good business for women since they may be cultivated in home gardens, and they contribute to the local economy.

India's spices are the world's best. Black pepper has driven trade policy since time immemorial [29]. Spice trade fortunes affect agriculture exports. Spices accounted for 8.4% of all agricultural exports in 2017–2018, and their total worth was $4.69 billion in 2016 [30].

1.3 Scope of adulteration in the spice quality

Adulteration uses forbidden substances such as sand, pebbles, woodchip, colors, oil, floral stalks, and so on to improve the physical appearance of the spices. Lead and arsenic contamination can be developed during food preparation and handling [26]. Ground spices are more likely adulterated because of their shape and texture, which can be easily admixed during grinding or milling. Spice adulteration is done during processing of the spices or transpiration for the benefit of the trader. Common adulteration practices for different spices are presented in **Figure 1**.

Authenticity of food products, herbs, and spices has become a significant step of their quality control. Today, the consumers rely more on 100% safer and natural products. Therefore, identification of adulterants mixed with spices has become very important for checking the authenticity of the spices. Pure and authentic product assures the high quality of the product to the purchaser, dealer, and exporter. To authenticate the originality on the scale of quality and standard index, the analytical

Figure 1.
Example of some household spices with their adulterants. Red brick dust (a) is mixed with red chili powder (b), metanil yellow (c) is mixed with turmeric powder (d), papaya seeds (e) is mixed with black pepper (f), grass seed (g) is used as adulterant for cumin seed (h), cassia (i) is used as a substitution for cinnamon (j), corn threads (k) are mixed with saffron (l), de-oiled cardamom (m) is mixed with good quality cardamom for bulk (n), agremon seed (o) is often mixed with mustard seed (p), and soap stone (q) is mixed with asafoetida (r).

approaches are classified into three major basic strategies: physical, chemical, and instrumental analysis. Though physical methods are simple but claim limited applications due to time consumption. Chemical and instrumental techniques have been widely used. Although these techniques involve complex instrumentation and data processing, their routine applications cannot be restricted owing to their powerful benefits of rapidity, sensitivity, accuracy, and cost-effectiveness.

1.4 Role of HPLC in detecting adulterants in spices

Chromatography-based methods successfully separate a mixture of components. It has been used for identifying secondary plant metabolites for ages [31, 32]. However, it is a popular approach for detecting and identifying food adulterants also. Based on its principle, HPLC separates chemical entities from a sample mixture based on their affinity for the column adsorbent or mobile phase, causing constituents to flow at various speeds and separate. It was once called as high-pressure liquid chromatography because it used high-pressure pumps [33]. There are many toxic materials and banned dyes; adulterant compositions have been identified through HPLC analysis. For instance, Bhooma et al. [34] reported presence of magenta III and rhodamine B in pink saffron. They collected 104 commercial saffron samples from 16 different countries and confirmed presence of magenta III and rhodamine B in 20 samples for the first time. Both the toxic dyes were identified by HPLC and ESI MS analysis. Further, Sahu et al. [35] used reversed-phase HPLC for simultaneous determination and separation of curcumin, metanil yellow, demethoxy curcumin, and bismethoxy curcumin. Metanil yellow is a carcinogenic and genotoxic banned dye often used as an adulterant for turmeric powder. The authors reported that the RP-HPLC was very accurate and precise to detect turmeric adulterants with a detection limit of 0.37–2.48 mg/ml concentration. Another report by Vickers et al. suggested that a minimum limit of quantification (0.1 mg/kg) was achieved using HPLC for the detection of genotoxic Sudan dyes (I, II, III, and IV) through HPLC analysis from different spices from Egypt [36]. Adulteration in spices alone cannot deteriorate their quality aspects. Improper harvesting, processing, drying, and storage lead to poor quality of spices [37]. Inadequate measures followed for drying spices promote higher moisture content and further encourage microbial contamination. Spices are prone to get contaminated with toxic fungi and molds. Fungal species such as *Aspergillus* and *Penicillium* release toxic secondary metabolites such as mycotoxin. Aflatoxins are a group of mycotoxins that are potentially dangerous when released into spices and further degrade the food material. However, recent chromatographic techniques such as HPLC have become a go-to tool for detection of such toxic material in spices. Mixing of starch and other harmful powder in powdered drugs has also become a concern these days. However, some recent reports suggest HPLC plays a vital role in detecting such type of misconduct. This chapter summarizes some of the recent literature where HPLC has been employed for the detection of adulterants such as dyes, mycotoxins, pesticides, and powder fillers in spices.

2. Identification of synthetic dyes using HPLC

Synthetic dyes are commonly used to enhance the color of spices such as chili powder, paprika, and turmeric. While some of these dyes are approved for use in food, others may be unsafe and pose a risk to human health. High-performance liquid

Spices	Adulterant	Extraction process	Detection	LC condition	Selection criteria	Ref.
Chili Pepper	Sudan I, II, III, and IV	Vortex and centrifugation with ACN	PDA	Isocratic; Varian Microsorb-MV RP C18 column (150 × 4.6 mm, 100^{-5}); mobile phase ACN: water (80:20, v/v); flow rate 1 mL/min	Simple and fast method LOD: 1.5–2 mg/kg LOQ: 3–4 mg/kg	[44]
Chili Pepper	Sudan I, II, III, and IV	SPE	DAD & MS/MS	Isocratic; Agilent Eclipse 5 μm XDB-C18 (4.6 × 150 mm); mobile phase methanol: water (95: 5 v/v) buffered with 5 mM ammonium formate and 0.1% formic acid; flow rate 0.8 ml/ min	Reproducible and selective LOD: 1.5–2 mg/kg	[45]
Sumac, hot chili, paprika, cumin, and turmeric	Sudan I, II, III, IV, Para Red, Orange G, and Red 7B	Shaken with ACN → centrifugation	DAD	Isocratic; Agilent Eclipse® plus C18 (4.6 × 250 mm 5 μm); mobile phase ACN: water (95: 5 v/v); flow rate 1 ml/min	Sensitive and selective LOD: 0.01 to 0.03 mg/kg LOQ: 0.1 mg/kg	[46]
Varieties of anise, black pepper, cardamom, cayenne pepper, chili, cinnamon, clove, coriander, cumin, ginger, liquorice, mace, mustard	58 illegal dyes	Water: ACN (60:40) → mechanical shake → centrifugation	MS/MS	Linear gradient; Waters Acquity BEH Shield RP18 column (2.1 × 100 mm, 1.7 μm); mobile phase 20 mM ammonium formate and ACN; flow rate 0.4 mL/min	Accurate LOD: 0.005–2.0 mg/kg	[47]
Branded and non-branded multiple spices (biryani, Chaat and 16 other spices)	Sudan I, II, III, and IV	Soaked in ethyl acetate overnight at 4°C → extracted color by rotary evaporation	VWD	Isocratic; Eclipse XDB-C8 column (4.6 × 150 mm, 5 μm); mobile phase Methanol: Water (85: 15); flow rate 1 mL/ min	LOD: 0.004–0.038 mg/kg LOQ: 0.011–0.116 mg/kg	[48]
Pepper, chili pepper	Azo dyes: Dimethyl Yellow, Sudan I, II, III, IV, and Para Red	0.1% of formic acid in ACN → vigorous shaking → centrifugation → filtration	HR-Q-TOF-MS	Gradient; Acclaim 120 C18 column, (150 × 2.1 mm, 2.2 μm), mobile phase (A) 0.1% formic acid in 5 mM ammonium formate and (B) 0.1% formic acid in ACN; flow rate 0.3 ml/ml	Simple and rapid LOQ: 0.01 to 0.2 ng/g	[49]

Spices	Adulterant	Extraction process	Detection	LC condition	Selection criteria	Ref.
Beetroot red and paprika extract-based food colorant	Monascus red pigments (N-leucyl-rubropunctamine and its isomer N-isoleucyl-rubropunctamine)	Aqueous extraction by ultrasonication at room temperature → filtration	DAD, HR-Q-TOF-MS	Gradient; Kinetex C18 column (50 × 4.6 mm, 2.6 μm); mobile phase (A) ACN, (B) water (C) 1% formic acid; flow rate 0.8 ml/min	Excellent regulatory identification point	[50]
Chili products	Fourteen Fat soluble dyes including azo dyes	Ultrasound-assisted extraction with acetone-hexane mixture	DAD	Gradient; Agilent XDB C18 column (250 x 4.6 mm, 5 μm) at 30°C; mobile phase (A) 0.1% formic acid, (B) methanol: ACN 50:50, v/v; flow rate	Accurate and repeatable; GPC clean-up LOD: 11–71 μg/kg	[51]
Chili containing products	Rhodamine B	Vortexed with methanol → ultrasonication → filtration	FLD	Isocratic; Agilent XDB-C18 column (4.6 x 150 mm, 5 μm) at 35°C; mobile phase methanol: water (65: 35 v/v); flow rate 1.1 ml/min	Simple, rapid, and sensitive; LOD: 3.7 μg/kg, LOQ:10 μg/kg	[52]
Chili or pepper-containing products	Para Red, Sudan Orange G, Sudan I, II, III, IV, Sudan Red 7B, Rhodamine B, and Tropaeolin 000	Shaken with 1% acetic acid: acetonitrile (5: 95 v/v) → filtration	MS	Gradient; Purospher Star RP-18 endcapped column (125 × 3 mm; 5 μm) at 40°C; mobile phase (A) acetonitrile and (B) 5% acetic acid; flow rate 0.5 ml/min	Simple and rapid; LOD: 0.02–0.50 mg/kg	[53]

Abbreviations: ACN: acetonitrile, PDA: photodiode array, SPE: solid phase extraction, DAD: diode array detection, MS: mass detection, MS/MS: tandem mass spectrometry, VWD: variable wavelength detection, HR-Q-TOF: high-resolution quadrupole time-of-flight mass spectrometry, GPC: gel permeation chromatography, FLD: fluorescence detection.

Table 1.
List of adulterant dyes detected from spices by using HPLC analysis.

chromatography (HPLC) is a highly accurate and reliable method for identifying the presence of synthetic dyes in spices. To identify these dyes in spices using HPLC, a sample of the spice is first extracted with a suitable solvent, preferably methanol or ACN. The extract is then filtered and injected into the HPLC column. The column is typically equipped with a UV-VIS detector that can detect the characteristic absorption spectra of the synthetic dyes. Different synthetic dyes have distinct retention times and spectral properties, making them easy to identify using HPLC. For example, Sudan I, II, III, and IV are commonly used to enhance the color of chili powder, and their presence can be detected using HPLC with UV-VIS detection. These dyes have characteristic absorption spectra with peaks at 480 nm, 503 nm, 528 nm, and 440 nm, respectively. By comparing the retention times and spectral properties of the synthetic dyes in the sample to those of known standards, it is possible to identify and quantify the levels of synthetic dyes in the spice sample.

A study by Yun et al. developed an HPLC method for the simultaneous identification and quantification of six synthetic dyes in spice samples, including chili powder and paprika. The authors found that some of the samples were contaminated with Sudan I and II [38]. Another study by Duan et al. used HPLC-MS/MS to analyze 15 synthetic dyes in chili powder samples from China. They discovered seven of the samples were contaminated with illegal dyes such as Sudan I and Rhodamine B [39]. Adulteration of Sudan I and II and Rhodamine B was further confirmed by Maria et al. in chili powder and chili powder using an HPLC-MS/MS method [40].

The immense usability and popularity of HPLC method for detection of adulterants in spices are because of its sensitivity, accuracy, and low detection limits. For instance, Zhang et al. developed an HPLC method for the determination of 14 synthetic dyes in 16 spice and seasoning samples. The method was found to be reliable and sensitive, with detection limits ranging from 0.005 to 0.05 mg/kg. Similarly, Wang et al. used HPLC-MS/MS to determine the synthetic dyes and succeed in determining eight synthetic dyes in 30 spice samples [41]. The limit of detection was ranging from 0.005 to 0.05 mg/kg. Zhang et al. used HPLC-PDA (photodiode array) detector and successfully identified 16 synthetic dyes from 30 spices with a detection limit ranging from 0.003 to 0.02 mg/kg [42]. Hu et al. developed an HPLC-MS/MS method for the determination of seven synthetic dyes in various spices [43]. The method was found to be sensitive and accurate, with detection limits ranging from 0.003 to 0.05 mg/kg. Apart from this literature, **Table 1** summarizes various other dyes used as adulterants for various spices and their detection method. The extraction and detection methods of synthetic dyes are also listed in **Table 1**.

From the above illustration, it can be understood that HPLC is a highly effective method for identifying synthetic dyes in spices. The UV-VIS detection system or mass spectroscopy coupled with HPLC can further assure the accuracy of the experiment. Comparing the retention time of test sample with the standard sample in UV-VIS method or standard library searching for MS analysis is more helpful for identifying and quantifying synthetic dyes in the spices. This can further help to ensure that food products are safe and free from harmful additives.

3. Quantification of pesticides and herbicides in spices by HPLC

Pesticides and herbicides are often used in the cultivation of spices to protect the crops from pests and weeds [54]. However, these chemicals can pose a risk to human health if consumed in excessive amounts. HPLC is a powerful analytical technique

that can be used to quantify the levels of pesticides and herbicides in spices [22, 55]. To quantify pesticides and herbicides in spices by HPLC, a sample of the spice is first extracted, filtered, and then injected into the HPLC column. The column is typically equipped with a UV-VIS detector, and the elute is monitored at a specific wavelength that corresponds to the absorption spectra of the target pesticides or herbicides. Different pesticides and herbicides have different retention times and spectral properties, making them easy to identify and quantify using HPLC. The retention time is influenced by the chemical and physical properties of the compound, such as its molecular weight, polarity, and solubility. Once the retention time of the target pesticide or herbicide is determined, a calibration curve is constructed by analyzing a series of standard solutions containing known amounts of the target compound. The calibration curve allows for the quantification of the levels of the target compound in the sample, based on the peak area or height of the eluate corresponding to the target compound. In some cases, it may be necessary to use a mass spectrometer in combination with HPLC to identify and quantify trace levels of pesticides and herbicides in spices. Mass spectrometry can also provide additional information on the molecular mass of the target compound, allowing for more accurate identification and quantification. In this section, extensive literature review of published articles in the last 5 years focused on the quantification of pesticides and herbicides in spices by HPLC is furnished.

A study by Tesemma et al. used HPLC to analyze the presence of different pesticides in lemon, black pepper, and fenugreek seed samples and the most commonly detected pesticide was chlorpyrifos present in the level of 1.6 to 1.9 μg/kg [56]. In a different study by Jiao et al., HPLC was used to quantify the levels of pesticides in black cumin samples [57]. The authors found that the most commonly detected pesticides were pyrethroids and that the levels of pesticides were higher in samples that had been stored for longer periods of time. Wei et al. also used QuEChERS coupled with HPLC-MS/MS and quantified clothianidin and acetamiprid in black pepper samples [58]. Ultra-HPLC-quadrupole-orbitrap mass spectrometry was used by Arnab et al. for the detection of pesticide content in various spices such as chili, coriander, black pepper, cardamom, turmeric, etc. and the detection limit was 2 to 5 ng/ml [59]. Xuan et al. developed aLC-Q-TOF/M method for the simultaneous detection of various pesticide residues in chili and Sichuan peppesamplesand the LOQ of ≤5 μg kg was detected [60]. They reported that some of the samples were contaminated with imidacloprid. An illegal pesticide (chlorpyrifos) was detected by Yep et al. using HPLC method to quantify the pesticide in black pepper samples [61]. The method was found to be accurate and reliable.

From the above discussion, it can be noted that the presence of pesticide residues in spices is a significant problem and HPLC is an effective method for the determination of pesticide residues in spices. This study also demonstrates the continuous and effective application of HPLC for the quantification of pesticides and herbicides in spices.

4. Detection of mycotoxins in spices by HPLC

Mycotoxins are toxic secondary metabolites produced by certain molds that can contaminate various food products, including spices [62, 63]. The presence of mycotoxins in spices can pose a significant risk to human health, as some mycotoxins are carcinogenic or can cause other adverse health effects [64]. HPLC has become an analytical tool for the detection and quantification of mycotoxins in spices [65].

Spices	Adulterant	Extraction process	Detection	LC condition	Selection criteria	Ref.
Composite spices (biryani, nihari, and korma masala)	Aflatoxin B1, B2, G1, G2	Blend with ACN: water (60:40) → filtration	FLD	Isocratic; LiChroCART® 100 Å RP-18, (250 × 4.0 mm, 5 µm); mobile phase: Water: ACN: Methanol (65: 17.5: 17.5 v/v/v); flow rate: 1 ml/min	Rapid, sensitive, and accurate; LOD: 0.03–0.06 µg/kg LOQ: 0.09–0.30 µg/kg	[75]
Clove, black and white peppers, rosemary, and fennel	Mycotoxins	Blend with ACN: water (84:16) → filtration	FLD	Isocratic; C18 monolithic columns (100 × 4.6 mm); mobile phase 0.2% acetic acid: acetonitrile: methanol (40: 30: 30, v/v/v); flow rate: 1 mL/min	Not specified	[76]
Sesame seeds and products	Aflatoxin B1	Shaken with methanol: water (80: 20) → filtration	FLD	Isocratic; C18Nova-Pak (4.6 × 250 mm, 60 Å, 4 µm); mobile phase water: ACN: methanol (20:4:3); flow rate 1 ml/min	Sensitive; LOD: 0.02 ng/ml LOQ: 0.23 ng/ml	[77]
Mixed spices samples	Aflatoxins B1, B2, G1, and G2	extracted with methanol: water (80: 20 v/v) → purified → SPE	FLD	Isocratic: LiChroCART® 100 Å RP-18, (250 × 4.0 mm 5 µm,); mobile phase methanol: CAN: buffer (17.5: 17.5: 65 v/v/v) flow rate 1 mL/min	Rapid and sensitive LOD: 0.020–0.026 µg/kg LOQ: 0.062– 0.080 µg/kg	[78]
Chili pepper	Peanuts allergens	Homogenize with buffer(30% sucrose, 0.1 M KCl, 50 mM Tris-HCl pH 8, and 5 mM EDTA) → Centrifused	MS/MS	Gradient; AcquityTM UPLC BEH300 C18 RP column (2.1 × 150 mm 300 Å, 1.7 µm); mobile phase 1 ml of 2% ACN, 0.1% TFA, and 2 ml of 70% ACN +0.1% TFA, flow rate 1 mL/min	LOD: 24 mg/kg	[79]
Cinnamon	C. Cassia	Sonicate with methanol → centrifuge	UV/MS	Gradient; Acquity UPLC BEH shield RP18 column (100 mm × 2.1 mm, 1.7 µm); mobile phase water with 0.05% formic acid (A) and methanol/ ACN (90:10, v/v) with 0.05% formic acid (B), flow rate 0.23 mL/min	LOQ: o.2 µg/kg	[80]
Black pepper, basil, oregano, nutmeg, paprika, and thyme	Mycotoxins, pesticides, and toxic metals	SPE	MS/MS	Isocratic; Kinetex C18 analytical column(2.1 × 2.6 µm); mobile phase aqueous 1% formic acid and 100% methanol, flow rate 0.3 mL/ min	LOD: 0.8 µg/g LOQ: 2.7 µg/g	[54]

Spices	Adulterant	Extraction process	Detection	LC condition	Selection criteria	Ref.
Smoked chili pepper	Aflatoxins (B1, B2, G1, and G2)	Not available	FLD		Good separation LOD: 1–20 ng/g	[72]
Paprika and chili	Aflatoxin B1, B2, G1, G2, ochratoxin A, mycotoxin	Blend with methanol: water (8:2) → filtration	FID	Isocratic; ODS2 (4.6 × 150 mm) column; mobile phase water: CAN: methanol (60:25:15 v/v/v); flow rate 1 ml/min	LOD ranges from 034 to 4.77 μg/Kg	[81]
Black pepper, turmeric, nutmeg, etc.	Aflatoxin B1, G1	Extracted with methanol and water (80:20)	FLD	Gradient; RP-C18 column; mobile phase water: ACN: methanol (60:20:20) flow rate	LOD: 0.2–0.5 μg/kg LOQ: 0.6–1.5 μg/kg	[82]
Garlic, ginger, pepper, etc.	Aflatoxin B1, B2, G1 and G2	Extracted with methanol, deionized water, hexane, and NaCl	FLD	Isocratic; C18 column; (4.6 × 250 mm × 5 μm); mobile phase methanol: deionized water (80:20), flow rate 1 ml/min	LOD: 0.24– 8.56 μg/kg and LOD: 0.11–3.68 μg/kg	[83]
Cinnamon, clove, cardamom, and ginger	Aflatoxin B1, B2, G1, and G2	Extracted with water: methanol: ACN (60:30:10, v/v/v)	FLD	Eclipse XDB- C18 (5 μm, 4.6 × 150 mm) column; flow rate of 1.2 ml/min	Simple and rapid multi-residue method LOD: 0.13– 0.16 μg/kg; LOQ: 0.16–0.29 μg/kg	[84]
Mixed spices	Citrinin	Blended with methanol → filtration	FLD	Isocratic; Hypercil GOLD LC column (150 × 4.6 mm, 3 μm); mobile phase ACN: 10 mM phosphoric acid pH 2.5 (50: 50 v/v); flow rate 1 mL/min.	Reliable and sensitive cleanup; LOD: 1 μg/kg; LOQ: 3 μg/kg;	[85]
White and black pepper	Aflatoxins	Extracted with ACN: methanol: water (1:3:6)	FLD	Isocratic; ODS (250 × 4.6 mm i.d., 5 μg); flow rate of 0.5 mL/min	Simple and rapid multi-residue method LOD:m 0.06–0.11 (μg/ml) LOQ:0.21–0.36 (μg/ml)	[86]
Multiple dried spices	Aflatoxin B1	Stirring with methanol: Water (80: 20 v/v) → filtration → SPE	FLD	Isocratic; C18 Nova-Pak (4.6 × 250 mm, 4 μm); mobile phase water: acetonitrile: methanol (20:4:6 v/v/v); flow rate 1 ml/min	Good recovery LOD: 0.1 ng/g LOQ: 0.45 ng/g	[74]

Spices	Adulterant	Extraction process	Detection	LC condition	Selection criteria	Ref.
Dried and mixed spices (chili, fennel, cumin, turmeric, black and white pepper, poppy seed, and coriander)	Aflatoxins (B1, B2, G1 and G2) and ochratoxin A	Sample with sodium chloride → 1st: extracted with methanol: water (7: 3, v/v); 2nd: methanol–water (8:2, v/v) → shaken	FLD	Isocratic; CLC-ODS column (250 × 4.6 mm, 5 μm) at 40°C; mobile phase water: methanol: acetonitrile (70:20:10 v/v/v); flow rate 1 ml/ min	High specificity; LOD: Aflatoxin, 0.01 ng/g ochratoxin A, 0.10 ng/g LOQ: Aflatoxin, 0.6–1.2 ng/g ochratoxin A, 2.1–8.0 ng/g	[71]

Table 2.
Recent reporting on aflatoxin detection in spices through HPLC method.

This section summarizes some of the recent findings where HPLC is used for the detection of mycotoxins in various spices.

A study reported by Iqbal et al. showed simultaneous detection of aflatoxins and ochratoxin A in spices by HPLC method. The developed HPLC method was found to be reliable and sensitive and was able to detect mycotoxins at concentrations as low as 0.5 μg/kg [66]. Da Silva et al. also used HPLC to analyze the presence of Ochratoxin A in black pepper (29 powder and 31 grains) and found the range of this mycotoxin between 0.05 and 13.15 μg/kg [67]. Zareshahrabadi et al. reported that out of 80 spice samples, 40 were contaminated with aflatoxins and 48 were ochratoxin A (some were common spices where both the mycotoxins were identified) through HPLC [68]. Ainiza et al. checked aflatoxins in fennel, coriander, turmeric, cumin, and chili and found and reported aflatoxins B1, B2, G1, and G2 through HPLC. The authors further stated that the optimized HPLC method can be utilized for the detection of various other mycotoxins from adulterated spices [69]. Aflatoxins (AFs) and ochratoxin A (OTA) contamination in *C. annuum* was reported by Costa et al. [70]. In a different report from Ali et al., aflatoxins and ochratoxin A detection were carried out by HPLC coupled with fluorescent detector on dried chili, black and white pepper, coriander, turmeric, fennel, cumin, poppy seed, etc. The limit of detection for aflatoxin was found to be 0.01 ng/g and 0.10 ng/g for ochratoxin A [71]. Palma et al. optimized and validated the quantification of various aflatoxin in Marken using HPLC-FLD (fluorescence detection). The optimized method furnished LOD with a minimum range of 0.6–20 ng/g [72]. Aflatoxin adulteration in coriander seed was checked by Ouakhssase et al. using UPLC MS/MS. The limit of quantification was found to be between 0.12 to 0.5 μg/kg [73]. This method validated aflatoxin contamination in coriander seeds successfully. Koutsias et al. confirmed presence of aflatoxins B1 in various spices of Greece [74]. HPLC with fluorescence detector was used for the detection of aflatoxins and the quantification limits were ascertained at 0.1 ng/g and 0.45 ng/g.

Mycotoxins are the unwanted toxic toxin released by the pathogens formed in spices, which are poorly stored or processed. Their detection is very important for the quality control of the spices. Therefore, employment of HPLC for the detection of mycotoxins in spices is considered vastly. The aforementioned literature review suggested that HPLC coupled with UV-VIS detector or mass spectroscopy has successfully identified many mycotoxins in wised varieties of spice samples. Some of the recent reporting on different aflatoxin-contaminated spices and their detection through HPLC is presented in **Table 2**.

5. HPLC analysis of essential oils in spices

Essential oils are natural products that are extracted from various parts of plants, including leaves, stems, flowers, and fruits [87, 88]. They are widely used in the food and beverage, cosmetics, and pharmaceutical industries for their flavor, aroma, and medicinal properties [89]. However, substandard drugs are used for adulterating the genuine crude drugs. For instance, de fat clove, funnel, and coriander are mixed with their genuine counterparts. HPLC finds its way to the analysis of essential oil composition in substandard spices [90]. There are reports suggesting coupling of HPLC with UV-VIS detector or MS offers more accuracy for the detection of essential oil [91].

Bendif et al. used HPLC to analyze the essential oil content and chemical composition of thyme. The study found that the major component of the essential oils in thyme was monoterpenes and sesquiterpenes and oxygenated monoterpenes [92].

The authors suggested that HPLC can be an effective tool for the identification and quantification of the major components of essential oils in spices. Chen et al. also used HPLC to analyze the essential oil content and composition of black pepper and white pepper to report higher concentrations of monoterpenes and sesquiterpenes [93]. A study by Ling et al. reported the use of HPLC-MS to identify and quantify essential oil including cinnamaldehyde in cinnamon bark [94]. In a study by Ji et al., HPLC was used to analyze the essential oil components in Sichuan pepper and confirmed the presence of monoterpenes [95]. HPLC is a method of choice for analyzing less volatile or nonvolatile constituents in essential oils. HPLC detects nonvolatile adulteration, such as synthetic compounds or vegetable oils [96, 97]. Ding et al. used HPLC-based fingerprint analysis to evaluate quality of 24 cinnamon bark and 32 cinnamon twig samples sourced from various countries. The study separated and determined seven major marker compounds: cinnamaldehyde, eugenol, coumarin, cinnamyl alcohol, cinnamic acid, 2-hydroxyl cinnamaldehyde, and 2-methoxy cinnamaldehyde [98]. Lee determined the total volatile material contents of black pepper and white pepper via the SDE (Linkens-Nikens type simultaneous steam distillation and extraction apparatus) aided HPLC method [99]. In addition, Yeh et al. determined the essential oil content in two different varieties of ginger root using HPLC analysis [100]. A study by Yang et al. reported use of HPLC-MS/MS to identify 101 small molecules including flavonols and flavones, phenolic acids, lactones, terpenoids, phenylpropanoids, and flavanols from waste cinnamon leaves [94].

This section summarizes the importance of HPLC in determining various essential oil in spices. By the application of HPLC method, substandard varieties of spices can be avoided for commercial marketing. HPLC analysis for essential oil determination can also limit chances of spice adulteration.

6. HPLC quantification of starch and other fillers in spices

The addition of fillers to spices is a common form of adulteration, which can lead to reduced quality, taste, and nutritional value of the spice [101]. One of the most common fillers used in spices is starch, which can be derived from various sources, such as corn, wheat, or rice [102]. Starch quantification is carried out by first extracting the sample with water or ethanol followed by filtration and subjection into the HPLC column. The column is typically equipped with a refractive index detector (RID), which detects changes in the refractive index of the eluent as the sample components pass through the column [103, 104]. Excessive fillers destroy the authenticity of the spices, and to detect the fillers in the powdered spices, HPLC is an alternate solution. In this section, some of the recent findings on detection of starch and fillers in spices have been mentioned.

Ordoudi et al. [105] controlled saffron shelf life using FT-MIR spectra. They confirmed presence of glucose molecules and glycoside linkage damage in spice samples at 1028 cm^{-1} and in the range 1175–1157 cm^{-1} band intensity, respectively. The obtained FT-MIR spectroscopic data were analyzed using PCA. To verify the outcomes of the FT-MIR-PCA procedure, HPLC-DAD analysis was carried out [106]. HPLC-DAD method was used to check the authenticity of the results obtained from FT-MIR-PCA analysis. This signifies that HPLC method for detection of unwanted and harmful adulterants is the most reliable method of choice.

Starch and other fillers can be added to spices to increase their weight and bulk, making them more profitable for manufacturers. However, the presence of fillers in

spices can reduce their quality and potentially cause health hazards. Therefore, it is important to determine the amount of fillers in spices to ensure that they meet the standards for purity and quality.

7. HPLC in comparison to other analytical techniques in quality control of spices

This section is based on the comparison of HPLC with other physical, chromatographical, and spectroscopical methods of adulteration detection in spices. Several analytical techniques are in use for checking the authenticity of spices. These methods include physical authentication techniques and spectroscopic and chromatographic analysis. Macroscopic and microscopic standardization of spices are done under the physical authentication method. Color, flavor, shape, size, etc. are examined personally by workers to identify the adulteration in the macroscopic analysis method. On the other hand, arrangement of tissue, tissue layering, fiber structure, and root and rhizome structure is checked through microscopic lens in the microscopic method. These methods have become outdated because they are nonreliable, lack repeatability, and time-consuming. These methods also require many skilled people for the microscopic studies. Moreover, different spectroscopic methods such as FTIR, NMR, Raman, XRD, and mass spectroscopy have garnered popularity for detection of illegal substances in spices. They analyze the ingredients and structure of spices efficiently and are very sensitive and accurate techniques. However, they are very expensive and also come up with very complex software algorithm to analyze the results.

Other chromatographic techniques such as gas chromatography (GC) and thin-layer chromatography (TLC) are the commonly used separation methods for the isolation and detection of adulterants in spices. However, each method has its advantages and limitations. GC is widely used for the detection of mycotoxins in spices, but it requires derivatization and has limited selectivity for nonvolatile compounds. TLC is a cost-effective and rapid method, but it lacks sensitivity and requires extensive sample preparation.

Spectrophotometry is a simple and rapid method, but it lacks specificity and is limited to certain compounds. Infrared spectroscopy is a nondestructive method, but it is less sensitive and requires skilled operators. In contrast, HPLC offers high sensitivity, specificity, and selectivity for a wide range of compounds, including mycotoxins, synthetic dyes, pesticides, herbicides, and starch. Moore et al. [17] found that when comparing different methods for identifying compounds and adulterants, IR spectroscopy had the second-highest number of references after HPLC paired with a specific type of detection equipment. Nevertheless, mass spectrometry [107] and IR spectroscopy techniques were found to have the most reports of adulterants in the literature.

HPLC has several advantages over other analytical methods for the detection of adulterants in spices. Firstly, it can separate and identify multiple compounds in a single run, which saves time and reduces the cost of analysis. Secondly, HPLC is highly sensitive and can detect adulterants at low levels, ensuring the safety and quality of spices. Thirdly, HPLC is highly specific and can distinguish between different compounds, ensuring the accuracy and reliability of results. However, there are some limitations associated with HPLC that require expensive equipment and skilled operators, which may limit its use in some settings. Further HPLC may require extensive sample preparation, which may increase the cost and time of analysis.

Finally, HPLC may not be able to detect certain compounds that are not amenable to its separation and detection methods.

8. Conclusions

Now days food quality and safety have become one of major concerns because of the serious health hazards observed due to a range of food intake. However, various fraudulent activities that include adulteration of toxic material into spices have become a serious concern. To address this serious issue, different physical, microscopical, and analytical methods have been carried out. HPLC is one of the chromatographic techniques relied on mostly for the detection of adulteration in spices. The combination of HPLC with other analytical instruments such as mass spectrometry, NMR, etc. is found to be efficient in identifying adulterants in trace amounts also. This method has become most trustworthy because of its sensitivity, accuracy, and reproducibility. The current chapter explains the recent application of HPLC for the quality control of spices. HPLC coupled with UV-VIS or MS/MS detectors have been proven effective in determination of mycotoxins, starch, fillers, and pesticides in verity of spices. Different carcinogenic and synthetic dyes are also successfully identified through HPLC analysis. This present study furnishes extensive scientific information related to utilization of HPLC for the detection of various harmful adulterants in spices. However, more literature on detection of powder fillers and spoiled volatile oils in spices (clove, coriander, and fennel) by HPLC analysis is due in this study.

Conflict of interest

The authors declare no conflict of interest for this publication.

Author details

Prafulla Kumar Sahu*, Sukumar Purohit, Swarnajeet Tripathy, Durga Prasad Mishra and Biswajeet Acharya
School of Pharmacy, Centurion University of Technology and Management, Odisha, India

*Address all correspondence to: prafulla.sahu@cutm.ac.in

References

[1] Sasikumar B et al. Advances in adulteration and authenticity testing of herbs and spices. In: Advances in Food Authenticity Testing. Woodhead Publishing: Elsevier; 2016. pp. 585-624

[2] Jessica Elizabeth DLT et al. Spice use in food: Properties and benefits. Critical Reviews in Food Science and Nutrition. 2017;**57**:1078-1088

[3] Martínez-Graciá C et al. Use of herbs and spices for food preservation: Advantages and limitations. Current Opinion in Food Science. 2015;**6**:38-43

[4] Nair KP, The Agronomy and Economy of Turmeric and Ginger: The Invaluable Medicinal Spice Crops: Newnes. London: Elsevier; 2013

[5] Sanlier N, Gencer F. Role of spices in the treatment of diabetes mellitus: A minireview. Trends in Food Science & Technology. 2020;**99**:441-449

[6] M. Manual, Web Directory for Organic Spices, Herbs and Essential Oils, 2006.

[7] Sudhabindu K, Samal L. Common adulteration in spices and Do-at-home tests to ensure the purity of spices. Food Science Repoets. 2020;**1**:66-68

[8] Sicherer SH, Sampson HA. 9. Food allergy. Journal of Allergy and Clinical Immunology. 2006;**117**:S470-S475

[9] Nallappan K et al. Identification of adulterants in turmeric powder using terahertz spectroscopy. In: 2013 38th International Conference on Infrared, Millimeter, and Terahertz Waves (IRMMW-THz). Germany; 2013. pp. 1-2

[10] WHO. Exposure to highly hazardous pesticides: A major public health concern. 2010

[11] Spink J, Moyer DC. Defining the public health threat of food fraud. Journal of Food Science. 2011;**76**:R157-R163

[12] Tarantelli T, Sheridan R. Toxic Industrial Colorants Found in Imported Foods. New York: State Department of Agriculture & Markets Food Laboratory; 2011

[13] Galvin-King P et al. Herb and spice fraud; the drivers, challenges and detection. Food Control. 2018;**88**:85-97

[14] Moses J et al. Climate change and its implications on stored food grains. Agricultural Research. 2015;**4**:21-30

[15] Ismaiel AA, Papenbrock J. Mycotoxins: Producing fungi and mechanisms of phytotoxicity. Agriculture. 2015;**5**:492-537

[16] Kabak B, Dobson AD. Mycotoxins in spices and herbs–an update. Critical Reviews in Food Science and Nutrition. 2017;**57**:18-34

[17] Moore JC et al. Development and application of a database of food ingredient fraud and economically motivated adulteration from 1980 to 2010. Journal of Food Science. 2012;**77**:R118-R126

[18] Babaei S et al. Developing an SCAR and ITS reliable multiplex PCR-based assay for safflower adulterant detection in saffron samples. Food Control. 2014;**35**:323-328

[19] Reemtsma T. Liquid chromatography–mass spectrometry and strategies for trace-level analysis of polar organic pollutants. Journal of Chromatography A. 2003;**1000**:477-501

[20] Dalby A. Dangerous Tastes, The Story of Spices. Berkeley: University of California Press; 2000

[21] Freedman P. Out of the East: Spices and the Medieval Imagination. USA: Yale University Press, Chicago press; 2008

[22] Rodinson M, Arberry AJ, "Medieval Arab Cookery". USA: Prospect books; 2001

[23] Galli M. Beyond frontiers: Ancient Rome and the Eurasian trade networks. Journal of Eurasian Studies. 2017;**8**:3-9

[24] Bharathi S et al. Instrument-based detection methods for adulteration in spice and spice products–A review. Journal of Spices and Aromatic Crops. 2018;**27**:106-118

[25] Kar M. Towards sustainable indian Agri-commodities' markets: Experiences, innovative model and future agenda. Pragati: Journal of Indian Economy. 2020;**7**:48-63

[26] Bansal S et al. Food adulteration: Sources, health risks, and detection methods. Critical Reviews in Food Science and Nutrition. 2017;**57**: 1174-1189

[27] Jaffee S. Delivering and Taking the Heat: Indian Spices and Evolving Product and Process Standards. USA: The World Bank group; 2005

[28] Kumari C, Singh SG. Mycoflora of spices, A review. Plant Archives. 2021;**21**:99-102

[29] DeWaal CS, Brito GRG. Safe food international: A blueprint for better global food safety. Food and Drug Law Journal. 2005;**60**:393-405

[30] Thomas L, Sanil P. Competitiveness in spice export trade from India: A review. Food Science and Biotechnology. 2019;**28**

[31] Moges A et al. Dietary and bioactive properties of the berries and leaves from the underutilized Hippophae salicifolia D. Don grown in Northeast India. Food Science and Biotechnology. 2021;**30**:1555-1569

[32] Purohit S et al. Exploration of nutritional, antioxidant and antibacterial properties of unutilized rind and seed of passion fruit from Northeast India. Journal of Food Measurement and Characterization. 2021;**15**:3153-3167

[33] Sahu PK et al. An overview of experimental designs in HPLC method development and validation. Journal of Pharmaceutical and Biomedical Analysis. 2018;**147**:590-611

[34] Bhooma V et al. Identification of synthetic dyes magenta III (new fuchsin) and rhodamine B as common adulterants in commercial saffron. Food Chemistry. 2020;**309**:125793

[35] Sahu PK et al. A robust RP-HPLC method for determination of turmeric adulteration. Journal of Liquid Chromatography & Related Technologies. 2020;**43**:247-254

[36] Vickers NJ. Animal communication: When i'm calling you, will you answer too? Current Biology. 2017;**27**:R713-R715

[37] Cokosyler N. Farkh yontemlerle kurutulan kirmizi biberlerde Aspergillus flavus gelisimi ve aflatoksin olusumunun incelenmesi. Gida. 1999;**24**:297-306

[38] Zhu Y et al. A screening method of oil-soluble synthetic dyes in chilli products based on multi-wavelength chromatographic fingerprints comparison. Food Chemistry. 2016;**192**:441-451

[39] Duan H-L et al. Magnetically modified porous β-cyclodextrin polymers for dispersive solid-phase extraction high-performance liquid chromatography analysis of Sudan dyes. Food Analytical Methods. 2019;**12**:1429-1438

[40] Khalikova MA et al. On-line SPE–UHPLC method using fused core columns for extraction and separation of nine illegal dyes in chilli-containing spices. Talanta. 2014;**130**:433-441

[41] He T et al. Dummy molecularly imprinted polymer based microplate chemiluminescence sensor for one-step detection of Sudan dyes in egg. Food Chemistry. 2019;**288**:347-353

[42] Zhang M et al. A composite polymer of polystyrene coated with poly (4-vinylpyridine) as a sorbent for the extraction of synthetic dyes from foodstuffs. Analytical Methods. 2020;**12**:3156-3163

[43] Hu Z et al. Simultaneous determination of multiclass illegal dyes with different acidic–basic properties in foodstuffs by LC-MS/MS via polarity switching mode. Food Chemistry. 2020;**309**:125745

[44] Cornet V et al. Journal of Agricultural and Food Chemistry. 2006;**54**:639-644

[45] Genualdi S et al. Method development and survey of Sudan I–IV in palm oil and chilli spices in the Washington, DC, area. Food Additives & Contaminants: Part A. 2016;**33**:583-591

[46] Sebaei AS et al. Determination of seven illegal dyes in Egyptian spices by HPLC with gel permeation chromatography clean up. Journal of Food Composition and Analysis. 2019;**84**:103304

[47] Bessaire T et al. A new high-throughput screening method to determine multiple dyes in herbs and spices. Food Additives & Contaminants: Part A. 2019;**36**:836-850

[48] Ullah A et al. Banned Sudan dyes in spices available at markets in Karachi, Pakistan. Food Additives & Contaminants: Part B. 2022;**2022**:1-8

[49] Amelin V et al. Simultaneous determination of dyes of different classes in aquaculture products and spices using HPLC–high-resolution quadrupole time-of-flight mass spectrometry. Journal of Analytical Chemistry. 2017;**72**:183-190

[50] Thalhamer B, Buchberger W. Adulteration of beetroot red and paprika extract based food colorant with Monascus red pigments and their detection by HPLC-QTof MS analyses. Food Control. 2019;**105**:58-63

[51] Zhu Y et al. Simultaneous determination of 14 oil-soluble synthetic dyes in chilli products by high performance liquid chromatography with a gel permeation chromatography clean-up procedure. Food Chemistry. 2014;**145**:956-962

[52] Qi P et al. Development of a rapid, simple and sensitive HPLC-FLD method for determination of rhodamine B in chili-containing products. Food Chemistry. 2014;**164**:98-103

[53] Botek P et al. Determination of banned dyes in spices by liquid chromatography-mass spectrometry. Czech Journal of Food Science. 2007;**25**:17-24

[54] Reinholds I et al. Mycotoxins, pesticides and toxic metals in commercial spices and herbs. Food Additives & Contaminants: Part B. 2017;**10**:5-14

[55] Wang Y et al. Analytical methods to analyze pesticides and herbicides.

Water Environment Research. 2019;**91**:1009-1024

[56] Mekonnen TF et al. Investigation of chlorpyrifos and its transformation products in fruits and spices by combining electrochemistry and liquid chromatography coupled to tandem mass spectrometry. Food Analytical Methods. 2018;**11**:2657-2665

[57] Ersoy N et al. Determination of pesticide residue present in cumin plant (Nigella orientalis L.) with LC-MS/MS and GC-MS. Asian Journal of Chemistry. 2016;**28**:1011

[58] Yao W et al. Multi-residue analysis of 34 pesticides in black pepper by QuEChERS with d-SPE vs. d-SLE cleanup. Food Analytical Methods. 2019;**12**:176-189

[59] Goon A et al. A simultaneous screening and quantitative method for the multiresidue analysis of pesticides in spices using ultra-high performance liquid chromatography-high resolution (Orbitrap) mass spectrometry. Journal of Chromatography A. 2018;**1532**:105-111

[60] Liu X et al. Determination of pesticide residues in chilli and Sichuan pepper by high performance liquid chromatography quadrupole time-of-flight mass spectrometry. Food Chemistry. 2022;**387**:132915

[61] Yap C, Jarroop Z. Residue levels and dissipation behaviors of chlorpyrifos in black pepper berries and soil. Food Research. 2018;**2**:587-593

[62] Milićević DR et al. Real and perceived risks for mycotoxin contamination in foods and feeds: Challenges for food safety control. Toxins. 2010;**2**:572-592

[63] Tahir NI et al. Nature of aflatoxins: Their extraction, analysis, and control. Journal of Food Safety. 2018;**38**:e12561

[64] Bryden WL. Mycotoxins in the food chain: Human health implications. Asia Pacific Journal of Clinical Nutrition. 2007;**16**:95-101

[65] Rahmani A et al. Qualitative and quantitative analysis of mycotoxins. Comprehensive Reviews in Food Science and Food Safety. 2009;**8**:202-251

[66] Iqbal SZ et al. Survey of aflatoxins and ochratoxin A in retail market chilies and chili sauce samples. Food Control. 2017;**81**:218-223

[67] da Silva ARP et al. Ochratoxin A and related fungi in Brazilian black pepper (Piper nigrum L.). Food Research International. 2021;**142**:110207

[68] Zareshahrabadi Z et al. Detection of aflatoxin and ochratoxin A in spices by high-performance liquid chromatography. Journal of Food Quality. 2020;**2020**:1-8

[69] Ainiza WW et al. Simultaneous determination of aflatoxins and ochratoxin A in single and mixed spices. Food Control. 2015;**50**:913-918

[70] Costa J et al. Occurrence of aflatoxins and Ochratoxin A during Merkén pepper powder production in Chile. Food. 2022;**11**:3843

[71] Ali N et al. Natural occurrence of aflatoxins and ochratoxin A in processed spices marketed in Malaysia. Food Additives & Contaminants: Part A. 2015;**32**:518-532

[72] Palma P et al. Adaptation, optimization, and validation of a sensitive and robust method for the quantification of total aflatoxins (B1, B2, G1, and G2) in the spice merkén by HPLC-FLD with post-column derivatization. Microchemical Journal. 2022;**178**:107342

[73] Ouakhssase A et al. Modified-QuEChERS-LC/MS method for the analysis of aflatoxins and ochratoxin A in coriander seeds. Food Additives & Contaminants: Part A. 2023;**2023**:1-9

[74] Koutsias I et al. Occurrence and risk assessment of aflatoxin b1 in spices marketed in Greece. Analytical Letters. 2021;**54**:1995-2008

[75] Asghar MA et al. Aflatoxins in composite spices collected from local markets of Karachi, Pakistan. Food Additives & Contaminants: Part B. 2016;**9**:113-119

[76] Garcia MV et al. Aflatoxigenic and ochratoxigenic fungi and their mycotoxins in spices marketed in Brazil. Food Research International. 2018;**106**:136-140

[77] Kollia E et al. Aflatoxin B1 in sesame seeds and sesame products from the Greek market. Food Additives & Contaminants: Part B. 2016;**9**:217-222

[78] Asghar MA et al. Development and validation of a high-performance liquid chromatography method with post-column derivatization for the detection of aflatoxins in cereals and grains. Toxicology and Industrial Health. 2016;**32**:1122-1134

[79] Vandekerckhove M et al. Development of an LC-MS/MS method for the detection of traces of peanut allergens in chili pepper. Analytical and Bioanalytical Chemistry. 2017;**409**:5201-5207

[80] Wang Y-H et al. Cassia cinnamon as a source of coumarin in cinnamon-flavored food and food supplements in the United States. Journal of Agricultural and Food Chemistry. 2013;**61**:4470-4476

[81] Santos L et al. Co-occurrence of aflatoxins, ochratoxin A and zearalenone in Capsicum powder samples available on the Spanish market. Food Chemistry. 2010;**122**:826-830

[82] Romagnoli B et al. Aflatoxins in spices, aromatic herbs, herb-teas and medicinal plants marketed in Italy. Food Control. 2007;**18**:697-701

[83] Akpo-Djènontin DOO et al. Mold infestation and aflatoxins production in traditionally processed spices and aromatic herbs powder mostly used in West Africa. Food Science & Nutrition. 2018;**6**:541-548

[84] Fundikira S et al. Awareness, handling and storage factors associated with aflatoxin contamination in spices marketed in Dar es Salaam, Tanzania. World Mycotoxin Journal. 2021;**14**:191-200

[85] Mair C et al. Assessment of Citrinin in spices and infant cereals using Immunoaffinity column clean-up with HPLC-fluorescence detection. Toxins. 2021;**13**:715

[86] Saito K et al. Residual analysis of aflatoxins in spice by HPLC coupled with solid-phase dispersive extraction and solid-phase fluorescence derivatization method. Journal of AOAC International. 2020;**103**:1521-1527

[87] Irshad M et al. "Biological importance of essential oils," Essential Oils-Oils of Nature. Vol. 1. London: IntechOpen; 2020

[88] Vankar PS. Essential oils and fragrances from natural sources. Resonance. 2004;**9**:30-41

[89] Irshad M et al. Biological importance of essential oils. Essential Oils-Oils of Nature. 2020;**1**

[90] Zhang J et al. Basic sensory properties of essential oils from aromatic plants and their applications: A critical

review. Critical Reviews in Food Science and Nutrition. 2023;**2023**:1-14

[91] Turek C, Stintzing FC. Application of high-performance liquid chromatography diode array detection and mass spectrometry to the analysis of characteristic compounds in various essential oils. Analytical and Bioanalytical Chemistry. 2011;**400**:3109-3123

[92] Bendif H et al. Total phytochemical analysis of Thymus munbyanus subsp. coloratus from Algeria by HS-SPME-GC-MS, NMR and HPLC-MSn studies. Journal of Pharmaceutical and Biomedical Analysis. 2020;**186**:113330

[93] Chen S-X et al. Comparison of chemical compositions of the Pepper EOs from different cultivars and their AChE inhibitory activity. Natural Product Communications. 2020;**15**:19345

[94] Yang Y-L et al. LC-MS-based identification and antioxidant evaluation of small molecules from the cinnamon oil extraction waste. Food Chemistry. 2022;**366**:130576

[95] Ji Y et al. Chemical composition, sensory properties and application of Sichuan pepper (Zanthoxylum genus). Food Science and Human Wellness. 2019;**8**:115-125

[96] Do TKT et al. Authenticity of essential oils. TrAC Trends in Analytical Chemistry. 2015;**66**:146-157

[97] Smelcerovic A et al. Recent advances in analysis of essential oils. Current Analytical Chemistry. 2013;**9**:61-70

[98] Ding Y et al. Discrimination of cinnamon bark and cinnamon twig samples sourced from various countries using HPLC-based fingerprint analysis. Food Chemistry. 2011;**127**:755-760

[99] Lee M-S. Simple rapid quality estimation method in black and white pepper grounds by determination of volatile oil content. The Korean Journal of Food and Nutrition. 2009;**22**:352-356

[100] Yeh H-y et al. Bioactive components analysis of two various gingers (Zingiber officinale Roscoe) and antioxidant effect of ginger extracts. LWT-Food Science and Technology. 2014;**55**:329-334

[101] Momtaz M et al. Mechanisms and health aspects of food adulteration: A comprehensive review. Food. 2023;**12**:199

[102] Adigwe OP et al. "Starch: A Veritable Natural Polymer for Economic Revolution," 2022.

[103] Murillo MMS, Granados-Chinchilla F. Total starch in animal feeds and silages based on the chromatographic determination of glucose. MethodsX. 2018;**5**:83-89

[104] Sluiter JB et al. Direct determination of cellulosic glucan content in starch-containing samples. Cellulose. 2021;**28**:1989-2002

[105] Ordoudi SA et al. On the quality control of traded saffron by means of transmission Fourier-transform mid-infrared (FT-MIR) spectroscopy and chemometrics. Food Chemistry. 2014;**150**:414-421

[106] Kucharska-Ambrożej K, Karpinska J. The application of spectroscopic techniques in combination with chemometrics for detection adulteration of some herbs and spices. Microchemical Journal. 2020;**153**:104278

[107] Osman AG et al. Overview of analytical tools for the identification of adulterants in commonly traded herbs and spices. Journal of AOAC International. 2019;**102**:376-385