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Research Article

Facile authentication of commercial vanilla extracts using simple chromatographic method

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Abstract
The preparation of natural vanilla extracts involves the extraction of vanilla beans in a water-alcohol mixture, a process that is both costly and labor-intensive, compounded by the limited availability of vanilla beans. Consequently, the production of synthetic vanilla extracts is prevalent, increasing the potential for adulteration and counterfeit products in the market. A rapid and sensitive high-performance liquid chromatography (HPLC) method has been developed for authenticating vanilla extracts, requiring minimal sample preparation and demonstrating an efficient runtime of 8 minutes with high precision and repeatability. This method was applied to 52 commercial vanilla extract products, comprising 32 liquids, 9 powder, and 11 paste formulations, which were further screened for coumarin presence. Among the samples, 23 were identified as authentic vanilla extracts, while 29 were determined to be synthetic flavorings, with four authentic samples containing ethylvanillin. No coumarin was detected in any of the samples. This HPLC method is well-suited for routine, high-throughput analyses, offering a reliable tool for laboratories requiring efficient screening of vanilla authenticity.

Keywords: *Vanilla planifolia*, flavour, adulteration, analysis, HPLC

Introduction

Vanilla is one of the world's popular flavour extracts obtained primarily from *Vanilla planifolia*, a tropical climbing orchid native to Mexico, but currently cultivated in many countries, predominantly in Madagascar, Indonesia, Tahiti, and Tonga. During the ancient time, vanilla was only used as an additive for chocolate, and now it is a flavour on its own [1]. Vanilla is one of the most widely used flavour resources in confectionery, food, and beverages. The high demand for natural vanillin far exceeds the supply from all sources.

Commercial vanilla is primarily made from three species which include *Vanilla pompona*, *Vanilla planifolia*, and *Vanilla tahitensis* and each species has its distinctive traits. The extract is produced by soaking vanilla beans in a mixture of water and ethyl alcohol. Mature vanilla bean pods develop the characteristic vanilla flavors by maturing for three to four months before being extracted. The whole process of producing natural vanilla extract can be costly and time-consuming, as it requires cultivating and processing vanilla orchids, which are primarily grown in tropical regions [2]. Vanilla bean supply is also limited since it can only be produced in a few countries, therefore is not enough to supply the whole globe. For this reason, synthetic vanilla extracts are widely used [3]. Synthetic vanilla extracts are less complex and usually contain vanillin, ethyl vanillin, and other related compounds that are prepared from inexpensive starting materials [4].

Vanilla beans contain about 200 components including vanillin, one of the main chemical constituents. Pure vanillin appears white or slightly yellow. Vanillin and a few other closely related compounds including vanillic acid, isovanillin, 4-hydroxybenzaldehyde, and 4-hydroxybenzoic acid give the extract its distinctive vanilla taste and aroma

[5]. Other than vanilla bean constituents, ethyl vanillin, and coumarin also give similar flavor characteristics [6]. The structures of compounds that have vanilla flavor are shown in **Figure 1**. Vanilla has a flavor profile similar to that of tonka beans, which are a more affordable alternative to vanilla beans. Tonka beans contain coumarin which is a natural substance that gives off a scent similar to vanilla [7].

Given the high cost of producing high-quality vanilla extracts, numerous attempts to adulterate this product have been reported [1,8]. A simple technique to adulterate vanilla extracts is to utilize synthetic vanillin, which can be made from lignin, eugenol, or guaiacol. Alternatively, poorer-quality synthetic compounds can be used to replicate real vanilla extracts at a reduced cost. For example, ethyl vanillin has been utilized as an alternative to vanillin since it can enhance the aroma and flavour of the vanilla extract while being less expensive [9]. Coumarin has been the most commonly used adulterant in vanilla extract, and it is obtained mostly from the tonka bean [7]. Coumarin, on the other hand, has been banned from use as a food additive since it has been demonstrated to produce hepatoxicity in animals [10] and is hazardous to humans [11].

Artificial vanilla flavourings often contain synthetically generated vanillin and/or ethyl vanillin dissolved in alcohol, propylene glycol, and glycerine. To improve the impression of vanilla flavour, some manufacturers have adulterated vanilla extracts with coumarin [7]. Coumarin has a sweet herbaceous odour and has been used to enhance flavour and scent in food, tobacco, and cosmetics, can be found in many plant species [12].

There is an increase in demand for pure vanilla extract as consumers are now more conscious of what they consume and are willing to pay more, prioritizing quality over quantity. For these reasons, the authentication of vanilla extracts is crucial. Vanillin content is an important quality parameter of vanilla extract [13]. Vanillin and ethyl vanillin contents are important quality parameters of imitation and fortified vanilla flavours. The presence of ethyl vanillin and/or coumarin is often an indicator of adulteration in products fraudulently represented as pure vanilla extract [14]. Some irresponsible manufacturers are taking advantage of the current trend by claiming and marketing artificial vanilla extracts as pure vanilla extracts on the market, which is misleading to the consumer [15]. Due to food safety concerns and the high demand for high-quality products, a reliable method for analysis of the vanillin and ethyl vanillin contents of natural and imitation vanilla extracts is needed.

This work aimed to develop a simple and rapid method for authenticating vanilla extracts, helping consumers become more aware of artificial vanilla extracts marketed as pure or natural.

Materials and Methods

Preparation of test samples

Nine vanilla extract products were in powder form, 11 were pastes, and 32 were liquid solutions. Sample preparation for powder extract included dissolving 1 g of sample with 100 mL methanol and sonicated for 30 minutes. The solutions were then diluted 10 times using the same solvent composition. For the liquid extracts, sample preparation consisted of pipetting 250 µL of vanillin extract into a 25 mL volumetric flask followed by the addition of methanol to volume and sonication of the flask. The pastes were prepared by sonicating 1 g of samples in 10 mL methanol for 10 minutes. The paste solutions were then treated similarly to the sample preparation for the liquid extracts. The samples were filtered with a 0.22 µm syringe filter into HPLC vials.

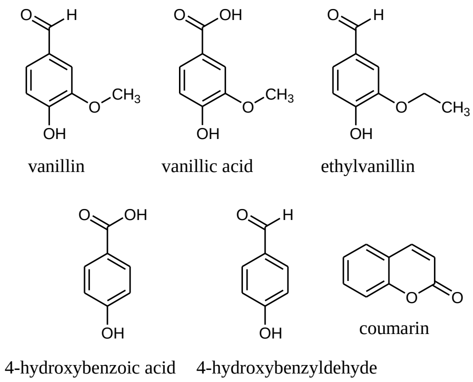

Figure 1. Structure of vanillin and other compounds with similar flavours and aroma

Preparation of reference standard solution

Standard solutions were prepared by weighing and dissolving the corresponding amount of the compound in methanol (Fischer Scientific, Waltham, MA, USA) followed by dilution to the appropriate working concentration (~1.0 mg/mL) in ultra-pure water purified at 18 MΩ.cm^{-1} by ELGA PURELAB® Option water purification system (Veolia Water Technologies, Paris, France.) and stored at 4°C. For long-term storage, the solutions were kept at -18 °C. To prepare the reference sample, 1 g of dried vanilla pods were cut into ~5 mm pieces and soaked in 100 ml methanol in a volumetric flask. The mixture was sonicated for an hour and 1 mL of the solution was diluted 10 times using methanol and then filtered with a 0.22 µm filter into a vial.

Preparation of sample solution

The dried plant samples (10.0 ± 0.5 g) were weighed and sonicated with 99.8% methanol for 1 hour to allow extraction of the quercetin compound. The solution was filtered using Whatman filter paper to produce a clear solution. Then, the solvent was evaporated using the rotary evaporator at 60 rpm to further concentrate the extract. The remaining sample extracts were weighed together with the round-bottomed flask and then the weight of the empty flask was subtracted to obtain the individual weight of the plant extracts. The extracts were then dissolved in 10 mL of 99.8% methanol.

Analysis of commercial vanilla products

The mass data was obtained from an LCMS system consisting of a Thermo Scientific™ Vanquish Horizon UHPLC hyphenated with Thermo Scientific™ Orbitrap Fusion™ mass spectrometer equipped with electrospray ionization (ESI) in negative mode. The instrument was externally calibrated with Thermo Pierce calibration solution prior to analyses. Full scan mode was used to record all the masses in the range of 200-2000 m/z. In addition to the full scan, data-dependent MS/MS fragmentation was recorded for the tallest peak on each spectral scan with various collision energies. The spectra data obtained from the LCMS analysis were viewed through FreeStyle™ 1.6 SPI software.

The chromatographic analyses were performed on the Agilent 1100 Series HPLC Value System from Agilent Technologies. A mobile phase consisting of acetonitrile and water (60:40, v/v) delivered by isocratic elution with the flow/rate of 1.0 mL/min. Separation made using ZORBAX StableBond Aq, 4.6 x 250 mm, 5 µm C18 column, UV detection at a wavelength of 280 and 340 nm, temperature maintained at 35 °C; The injection volume is 10 µL; Chromatographic run of 8 min; Injection procedure is blank (diluent), reference solution (three times), test solution, and blank solution to rinse.

Results and Discussion

Mass spectrometric analyses were performed to accurately identify chromatographic peaks associated with chemical components in vanilla pods, enabling precise identification of analytes in real samples. Initial chromatographic experiments on the LC-MS system generated a total ion chromatogram (TIC), which illustrated the elution sequence for key compounds, including vanillin, 4-hydroxybenzaldehyde, and their respective acids, vanillic acid and 4-hydroxybenzoic acid. The compounds were identified based on their mass-to-charge (m/z) values obtained from the mass spectra and further confirmed by MS/MS fragmentation patterns. **Table 1** provides a summary of the TIC data for vanilla pod extracts, including mass spectra and MS/MS values for each fragmented ion. **Figure 2** presents the most likely interpretation of MS/MS fragmentation, informed by detailed mass fragment spectra data. To date, no comparable studies are available for reference. Compound assignments were made according to their fragmentation profiles using the Compound Discoverer 3.1 software. This chromatographic profile of vanilla pod extract serves as a reference for authentic vanilla products.

The chromatographic profile of the vanilla pod extract was established using high-performance liquid chromatography with UV detection. **Figure 3(a)** displays the chromatogram, highlighting the primary compounds in the sample. Tentative identification of these peaks was achieved by comparing their elution order with those obtained via liquid chromatography-mass spectrometry (LC-MS). This profile served as a reference for verifying the authenticity of product samples, identifying vanillin as the major compound along with 4-hydroxybenzaldehyde, vanillic acid, and 4-hydroxybenzoic acid. Additional peaks observed in the chromatogram may correspond to other compounds in the extract that remain unidentified.

For comparison, the commercial vanilla flavour (Star brand), endorsed by the Malaysian government as safe for food use, was employed as a reference for artificial vanilla essence. **Figure 3(b)** shows the chromatogram of the Star brand vanilla flavour, displaying only two peaks associated with vanillin and ethylvanillin. While vanillin appears in both samples, the profiles differ notably, with a higher vanillin concentration in the pure vanilla extract compared to the artificial flavour. Additionally, the artificial flavour was found to lack coumarin, a compound prohibited in food products due to health risks.

Table 1. Mass data of vanilla bean pod extract

Peak No.	Retention Time (min)	Observed m/z	Molecular Ion Formula	Mass Fragments	Tentatively Assigned Substance
1	1.82	167.03497	$C_8H_7O_4^-$	153.01244 123.04629 109.02281	Vanillic acid
2	2.55	137.02446	$C_7H_5O_3^-$	121.03678 105.04186 93.11124	4-hydroxybenzoic acid
3	2.85	121.02955	$C_7H_5O_2^-$	105.12194 93.04255	4-hydroxybenzaldehyde
4	3.13	151.04018	$C_8H_7O_3^-$	137.02442 123.02950 109.05243 93.04186	Vanillin

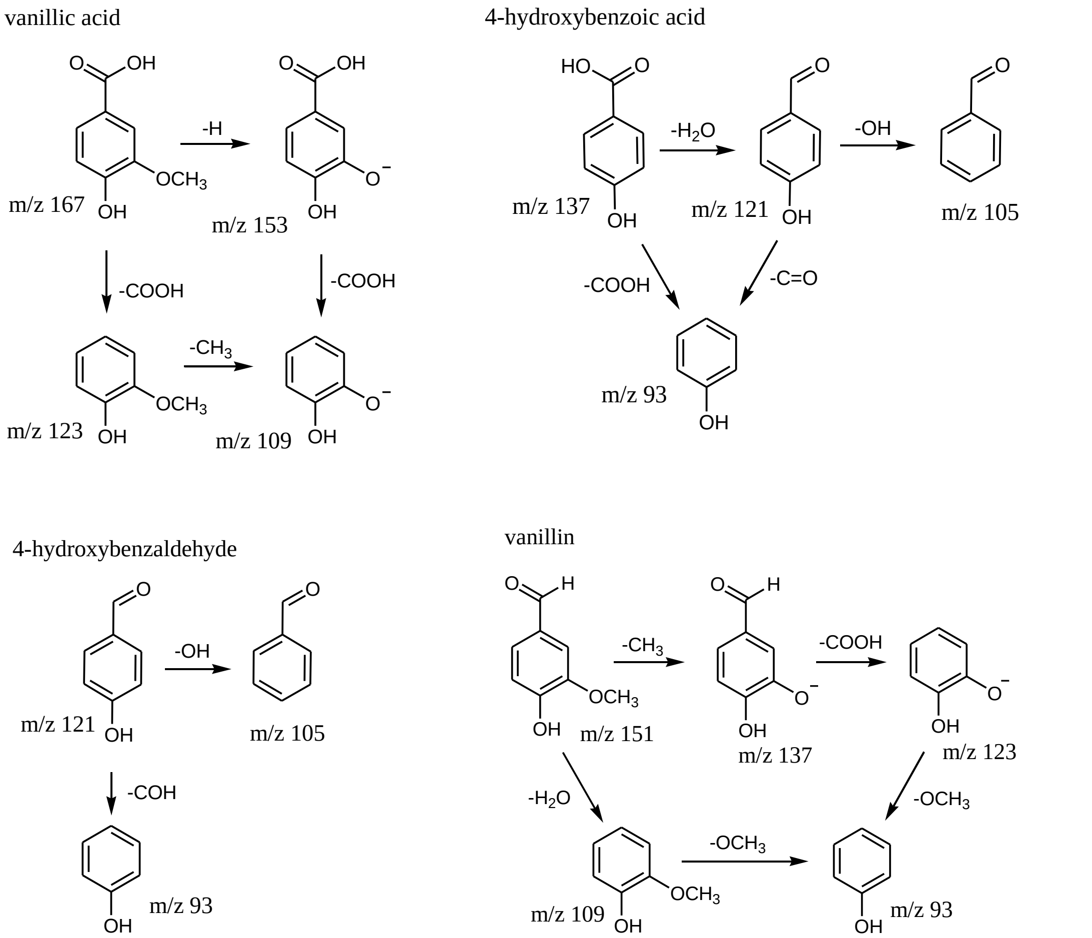

Figure 2. Proposed fragmentation pattern with tentative structures for the product ions of compounds detected in vanilla bean extract

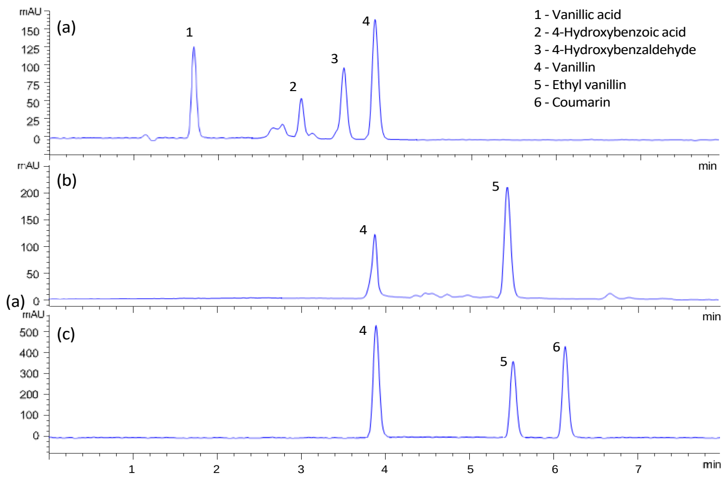

Figure 3. HPLC analyses of vanilla pod extract (a), artificial vanilla flavour (b), and standard mixture (c)

Natural vanilla extract is obtained from vanilla beans, which contain a complex blend of compounds that contribute to its distinctive flavour profile. Artificial vanilla extract, on the other hand, relies primarily on vanillin - the main flavour compound responsible for vanilla's characteristic taste and aroma. To create a more affordable and accessible alternative, synthetic vanillin is used to replicate the flavor of natural vanilla. Additionally, ethylvanillin, a fully synthetic compound with a flavor similar to vanillin, is often included to further reduce costs. Although artificial vanilla lacks the depth and complexity of natural vanilla, it delivers a comparable vanilla-like taste suitable for various food products and culinary applications.

Of the 52 vanilla extract products tested, less than half were identified as pure vanilla extract (**Table 2**). The analysis also revealed that a white powder marketed as pure vanilla was primarily sugar flavored with vanillin not derived from vanilla. Three samples of vanilla essence contained very low levels of vanillin, nearly at trace levels, prompting administrative measures to ensure compliance with labeling standards. Additionally, some samples labeled as "vanilla concentrate" were found to be vanilla flavoring diluted with water. Four samples of pure vanilla extract also contained ethylvanillin. Furthermore, some flavorings included aromatic caramel misrepresented as a flavoring carrier, when in fact, it served primarily as a coloring agent.

More than half of the natural vanilla flavours sampled were artificial. Still, most presented themselves as having a natural vanilla flavour. These flavourings in particular had only vanillin and ethylvanillin content. Whether the proportion of vanillin comes from vanilla extract or biotechnological processes, cannot be determined. All vanilla products labelled 'pure' tested complied with the claims on the label. Four authentic vanilla extracts contained a trace amount of ethylvanillin, which might be derived from vanillin suggesting improper storage of the products. None of the extracts tested, whether pure, artificial, or blended, tested positive for the banned substance coumarin.

Vanillin content in vanilla beans can vary based on factors like growing conditions, maturity, and post-harvest processing. To ensure a consistent and recognizable vanilla flavour in their products, manufacturers may add a small amount of synthetic vanillin, which closely mimics the taste of natural vanillin. Also, natural vanilla extract can be sensitive to environmental factors such as heat, light, and air, which may lead to flavour degradation over time. Synthetic vanillin exhibits higher stability and resilience to such factors, contributing to a longer shelf life for pure vanilla extracts. The inclusion of synthetic vanillin helps preserve the integrity of the vanilla flavour, ensuring that the extract remains delightful and consistent from the time of purchase to its consumption.

The analyses have therefore highlighted fraudulent practices aimed in particular at (1) replacing vanilla products with inferior products such as vanilla extracts consisting of water and artificial flavour, (2) intensifying the taste, smell, and appearance of vanilla by adding in vanilla flavoured aroma, (3) reproduce the vanilla flavour by adding vanillin from biotechnology in a significant proportion compared to the vanillin from vanilla pods, (4) standardize the color of vanilla flavours with caramel acting as a colouring agent which made the products are no more pure extracts.

Table 2. Detection of indicator compounds for authentication of vanilla products in 52 samples across 3 different categories, i.e. liquid vanilla extract, vanilla paste, and powder

Product Formation	No.	Label	Brand	Country of Origin	Chromatographic identification			
					Vanilla Bean Extract	Vanillin	Ethylvanillin	Coumarin
	Reference	Vanilla pod extract	-	Malaysia	✓	✓		
	Reference	Perisa Vanila / Artificial Vanilla Flavour	Star Brand	Malaysia		✓	✓	
Liquid	1	100% Pure Vanilla Extract	Qspice	Malaysia	✓	✓		
	2	Chef Amer Premium Essence	Chef Amer	Malaysia		✓	✓	
	3	Creamy Vanilla Flavour	Myflavor	Malaysia		✓	✓	
	4	Esen Vanilla Sedap Rasa Premium	Sedap Rasa	Malaysia		✓	✓	
	5	Finest Vanilla Madagascar	Queen	Australia	✓	✓		
	6	French Vanilla Extract	*[Repacked]*	-	✓	✓	~	
	7	Green House Vanilla Flavour	Green House	Malaysia		✓	✓	
	8	Harum Pure Vanilla Extract	Harum	Malaysia	✓	✓		
	9	Madagascar Bourbon Pure Vanilla Bean Extract	Nielsen-Massey	USA	✓	✓		
	10	Madagascar Pure Vanilla Extract	Simply Organic	USA	✓	✓		
	11	Master Blend Pure Vanilla Extract	Queen	Madagascar	✓	✓		
	12	Natural Vanilla Extract	*[Repacked]*	-	✓	✓	~	
	13	Organic Vanilla Bean Extract	Taylor & Colledge	Australia	✓	✓		
	14	Premium Silky Vanilla Flavour	Chef Amer	Malaysia		✓	✓	
	15	Premium Vanilla Essence	Dapur Desa	Malaysia		✓	✓	
	16	Premium Vanilla Essence	BWY	Malaysia		✓	✓	
	17	Pure Vanilla made from Tahitian Vanilla Beans	Uncang	Malaysia	✓	✓		
	18	Pure Vanilla Extract	Essential Everyday	USA	✓	✓		
	19	Pure Vanilla Extract	Mccormick®	USA	✓	✓	~	
	20	Pure Vanilla Extract Tahitian Pure Vanilla	Melpona	Indonesia	✓	✓		
	21	Rb Vanilla	CK	-		✓	✓	
	22	Scrumptious Vanilla Essence	Sedap Rasa	-		✓	✓	
	23	Vanilla Aroma	Siebin	Germany		✓	✓	
	24	Vanilla Essence	Flavors dotcom	Malaysia		✓	✓	
	25	Vanilla Essence	Bakez Grocer			✓	✓	
	26	Vanilla Extract with Vanilla Seeds	Vanilla Trading	Papua New Guinea	✓	✓		
	27	Vanilla Flavoring Essence	Rayner's	UK		✓	✓	
	28	Vanilla Flavour	Green House	Malaysia		✓	✓	
	29	Vanilla Flavour	Myflavour *[Repack]*	Malaysia		✓	✓	
	30	Vanilla Flavouring (Perisa Vanila)	Dapur Desa	Indonesia		✓	✓	
	31	Vanilla Paste Esen	AC Bakery	Malayisa		✓	✓	
	32	Vanilla Prima	PastryPro *[Repack]*	Germany		✓	✓	

Product Formation	No.	Label	Brand	Country of Origin	Chromatographic identification			
					Vanilla Bean Extract	Vanillin	Ethylvanillin	Coumarin
Paste	33	Creamy Vanilla Emulco	Azim Bakery	Malaysia		✓	✓	
	34	French Vanilla Extract	Mec3	-	✓	✓	~	
	35	Finest Madagascar Vanilla Bean Paste	Queen	Australia	✓	✓		
	36	La Dame in Vanilla	La Dame	Indonesia	✓	✓		
	37	Madagascan Vanilla Bean Paste with Seeds	Queen	Australia	✓	✓		
	38	Organic Vanilla Bean Paste with Vanilla Seeds	Queen	Australia	✓	✓		
	39	Vanilla Bean Extract	Healthy Baker	-		✓	✓	
	40	Vanilla Bean Paste with Seeds	Taylor & Colledge	Australia	✓	✓		
	41	Vanilla Emulco	Sinco	Mexico		✓	✓	
	42	Vanilla Paste	Flavors dotcom	Malaysia		✓	✓	
	43	Vanilla Paste	Vanilla Trading	Papua New Guinea	✓	✓		
Powder	44	Flavour Powder Vanilla/ Serbuk Perisa Vanilla	Trans	-		✓	✓	
	45	Prisa Vanilla Serbuk	Koepoe Koepoe	Indonesia		✓	✓	
	46	Serbuk Perisa Vanila	Bll	-		✓	✓	
	47	Vanili Bubuk	Penguin	Indonesia	✓	✓		
	48	Vanilla Bean Powder	Matakana	New Zealand	✓	✓		
	49	Vanilla Flavour Powder	Myflavor	Malaysia		✓	✓	
	50	Vanilla Powder Food Grade	Sinco	Malaysia		✓	✓	
	51	Vanilla Powder (Ground Bean)	Vanilla Trading	Papua New Guinea	✓	✓		
	52	Vanillie	Top Lace	Indonesia		✓	✓	

✓ present in product samples
~ detected at a trace amount

Conclusion

This study contributes to the establishment of easy HPLC conditions for rapid and sensitive authentication of vanilla extract and routine purpose analysis. A simple screening method for testing the authenticity of vanilla extracts was developed and performed on an ordinary HPLC system. It demonstrated excellent precision, repeatability, and carryover performance. Pure vanilla extracts contain a wide variety of compounds but mainly four major compounds, vanillic acid, 4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, and vanillin which is similar to the standard profile from the vanilla pod. Artificial vanilla essences tested contain ethyl vanillin as a major constituent, a significant amount of vanillin, and a few other constituents for flavour and aromatic enhancement.

A series of 52 commercial vanilla extracts were screened and all of the extracts labelled as pure, proved to be authentic extracts. The artificial vanilla flavours have different types of labelling. None of the extracts tested positive for the presence of coumarin. The investigation of product ingredients found that 13 percent of vanilla products in various formations tested were thinned with water, alcohol, sugar, or starch.

The presence of vanillin in pure vanilla extracts serves multiple purposes, ranging from flavour enhancement and consistency to cost-effectiveness and flavour stability. Synthetic vanillin, when used responsibly, can support the preservation of the vanilla flavour while making it more accessible to a broader audience. Nonetheless, it is crucial for consumers to be aware of product labelling and choose reputable products.

The HPLC system is an ideal solution for laboratories that require a simple-to-use analytical system for routine quantitative analysis. A further study can be done by using more variety of vanilla beans to compare their profiles. Furthermore, preparing a standard mix of vanillin and other compounds that may be detected in vanilla extracts such as ethyl vanillin, coumarin, vanillic acid, coumarin, and isovanillin, and comparing its profile with the profile obtained from the vanilla bean and the samples would also produce a more accurate result.

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