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Research Article

Analysis of Vanillin In Food Products By High Performance Thin Layer Chromatography

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ABSTRACT

A simple, fast, specific and precise high performance thin layer chromatography method has been developed for the estimation of vanillin in food products. Aluminium plates precoated with silica gel 60 GF 254 were used as stationary phase and a mixture of methanol-water-glacial acetic acid in the ratio of 20:05:02 (v/v) as mobile phase. Quantification was carried out by scanning the developed spots using a densitometer in absorbance mode at 275 nm. The Rf value of vanillin was 0.84. The results of the analysis have been validated statistically and by the recovery studies. Linearity was observed in the concentration range of 200-1000 ng/spot.

Keywords: Vanillin, HPTLC, food products

1. INTRODUCTION

Vanillin one of the most popular flavoring agents used in various food products, beverages and in perfume industry is a phenolics aldehyde, primarily obtained from the extracts of vanilla bean. It is also found in roasted coffee and Chinese red pine. It is chemically 4-Hydroxy-3-methyl benzaldehyde. With high demand for the supply of vanilla pods and the continued increase in their cost, artificial vanilla flavoring agents of synthetic origin are nowadays available.

Detailed review of literature for various analytical methods for the detection of vanillin revealed several methods based on different techniques. Viz RP HPTLC [1] densitometric method for the determination, validation of vanillin and related phenolics compounds in accelerated solvent extracts of *Vanilla planifolia.* A planar chromatographic method [2] was also reported for the determination of vanillin and ethyl vanillin flavorings. RP HPLC methods [3-5] were also reported for the determination of vanillin in *Vanilla plantifolia,* boiled peanuts and vanillin related aromatic compounds. A capillary electrophoresis micro chip approach with electrochemical detection [6] was also reported for the authenticity controlling of vanilla flavors. A paper chromatographic method [7] was also reported for the estimation of vanillin and ethyl vanillin in food stuffs. However there was no HPTLC method reported for the determination of vanillin in food products. The purpose of the present study was to evaluate vanillin content in vanilla flavored food products available in Indian market by an accurate and sensitive HPTLC method. The developed method was validated as per ICH guidelines [8].

2. MATERIAL AND METHODS

2.1. Solvents and chemicals

Vanillin was procured from sigma Aldrich limited, India. Food products were procured commercially. Chromatographic grade solvents like methanol, chloroform, acetone and glacial acetic acid were obtained from Qualigens chemicals, Mumbai, India.

2.2. Standard and sample solutions

Vanillin (100 mg) was accurately weighed into a 100 ml volumetric flask, dissolved in methanol and diluted up to the volume with same solvent. The stock solution was stored in light resistant containers. This stock solution was further used to furnish working standards.

Vanillins from three different samples (Vanilla essence, custard powder and vanilla flavoured ice cream) were extracted with methanol. 0.2 ml of vanilla essence solution (Solution I) was diluted to 10 ml with methanol. 1 ml of the above solution was again diluted to 10 ml with methanol and used for further analysis. 0.5 gm of samples (II and III) were extracted by adding 10 ml of methanol and sonicated for 15 min. The resulting solution was filtered using Whatmann filter paper No. 42 and used for further chromatographic analysis.

2.3. Chromatography

Chromatography was performed on an aluminum backed silica gel 60 GF_{254} TLC plates pre-washed with methanol. (0.2 mm Thickness).

Standard solutions of vanillin were prepared by transferring the stock solutions in different 10 ml volumetric flasks and diluted to the volume with methanol such that the concentrations are 200 -1000 ng/µl. The standards and three different sample solutions were applied to the TLC plates as 8.0 mm bands with 9.0 mm space between two bands using a Camag Linnomat IV sample applicator. The plates were developed with a mobile phase of Methanol: Water: Glacial acetic acid [20:05:02] in a TLC twin trough chamber.

After development the plates were dried at 60°C for 5 minutes and the quantification of the standards and samples were performed by means of a Camag TLC scanner III controlled by WinCATS 1.4.3 version software at 275 nm. The amount of vanillin in the sample solutions were computed from the calibration plot (Figure 1-4).

3. RESULTS AND DISCUSSION

The selected mobile phase of Methanol: Water: Glacial acetic acid [20:05:02] resolved vanillin efficiently and is shown in fig. I. The R_f value of vanillin was found to be 0.84. Vanillin solution gave an absorbance maximum at 275 nm and was selected for detection. The method was used to determine vanillin content in the food products selected for analysis. The results were tabulated in Table 1.

Table 1: Analysis of vanillin in food products

S. No	Sample	Amount of Vanillin present [mg ±RSD*]/ GM	Recovery [%±RSD*]
1	Vanilla Essence	48.60 ± 0.8475	99.35 ± 0.1490
2	Baker's Custard Powder	0.96 ± 0.1982	98.16 ± 0.2349
3	Vanilla flavored ice cream	2.23 ± 0.6863	98.32 ± 0.4351

**RSD of three determinations*

Table 2: System suitability studies

S. No	Parameters	Ellagic acid
1	Linearity Range	200 – 1000 ng/spot
2	Regression equation Y = mX+C	Y = 5.979X + 11516
3	Correlation coefficient	0.9971
4	LOD (ng)	1.05
5	LOQ (ng)	3.25

Table 3: Results from ruggedness studies*

Analysts	Amount recovered [%]		
	Vanilla Essence	Custard Powder	Vanilla Flavored Ice cream
Analyst I	99.35	98.16	98.32
Analyst II	99.95	99.46	100.23

** All the values are % recovery in food products*

Table 4 : Results from robustness studies

Development distance [mm]	Amount recovered [mg]		
	Strawberry fruit	Pomegranate fruit	Strawberry Jam
75.0	100.32	99.50	98.58
80.0	101.45	100.42	101.85

3.1. System suitability

System suitability tests are an integral part of a chromatographic analysis and should be used to verify that the resolution and reproducibility of the chromatographic systems are adequate for analysis. To ascertain the effectiveness of the method developed, system suitability tests were performed on a freshly prepared standard stock solution of vanillin.

3.2. Linearity

A calibration plot of peak area against concentration of vanillin was linear in the concentration range of 200 – 1000 ng/µl. The calibration lines were represented by the linear regression equation Y = 11516+5.978 X where Y is the peak area and X is concentration. The correlation coefficient r^2 was found to be 0.9971.

3.3. Limit of quantification and detection

The Limit of Quantification (LOQ) and limit of Detection(LOD) were calculated by use of the equations LOD=3 x N/B and LOQ = 10 x N/B where N is the standard deviation of the peak area of the drug, taken as a measure of the noise and B is the slope of the corresponding calibration

curve. The limit of quantification and the limit of detection for vanillin were found to be 1.05 ng and 3.25 ng respectively.

3.4. Accuracy and precision

The accuracy and precision of the method were studied by performing experiments by standard addition methods. Accuracy of the method was determined by recovery experiments. The recovery of the method was determined at single level by adding a known quantity of vanillin to the food products of pre analyzed samples and the mixtures were analyzed according to the proposed method. The average recovery obtained from each sample was between 98.16 and 99.35 % and is shown in table 1. From the data obtained, added recovery of standard vanillin was found to be accurate.

3.5. Ruggedness and robustness

Ruggedness is the measure of the reproducibility of a test result under normal, expected operating conditions from instrument to instrument and from analyst to analyst (Table 3). Robustness of the method was determined by making slight changes in the chromatographic conditions. No marked changes in the chromatograms demonstrated that the HPTLC method developed are rugged and robust (Table 4).

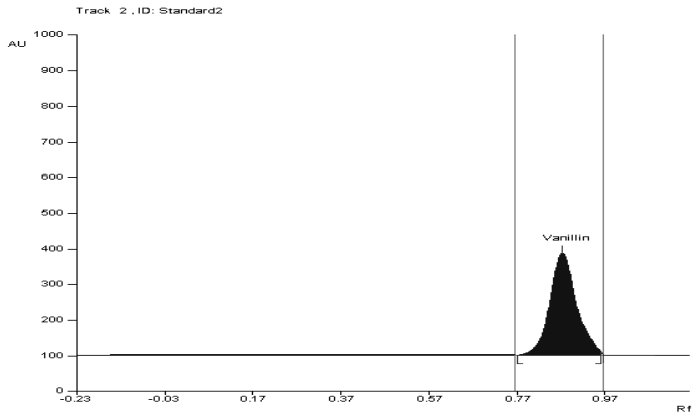

Fig. 1: Typical HPTLC Chromatogram of Standard Vanillin

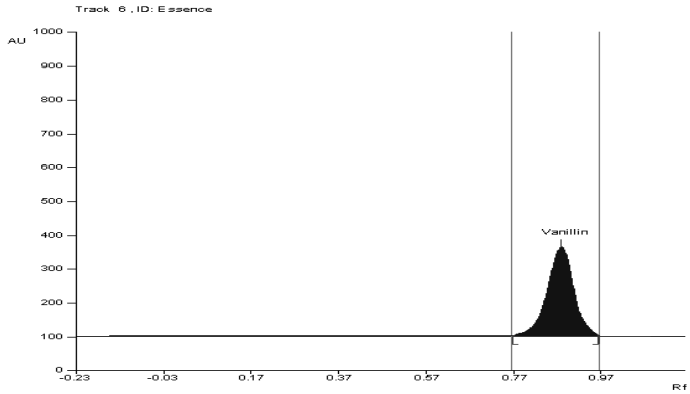

Fig. 2: Typical HPTLC Chromatogram of Vanillin in Vanilla Essence

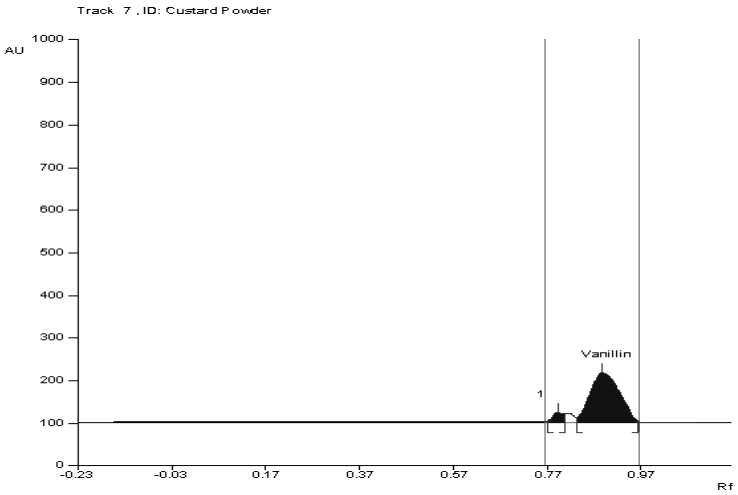

Fig. 3: Typical HPTLC Chromatogram of Vanillin in Baker's Custard powder

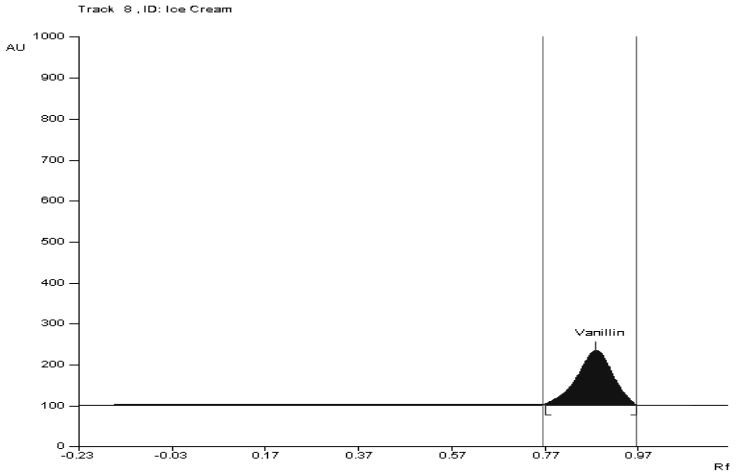

Fig. 4: Typical HPTLC Chromatogram of Vanillin in Vanilla flavored custard powder

4. CONCLUSION

The HPTLC method proposed for the determination of vanillin in three different food products were accurate, precise, rapid, selective and sensitive and therefore can be conveniently adopted for the routine analysis of vanillin in food products.

5. ACKNOWLEDGEMENT

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