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Spectrophotometric Analysis of Vanillin from Natural and Synthetic Sources

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A new spectrophotometric method has been examined for the determination of the flavouring agent vanillin (4-hydroxy-3-methoxy-benzaldehyde) (VAN) by derivatization with 1-aminonaphthalene. The derivative indicated molar absorptivity of 5540 L mol^{-1} cm^{-1} at 372 nm and obeyed Beer's law within 5-25 µg/mL. The colour reaction was highly stable and did not show any change in absorbance up to 24 h. The method was applied for the determination of vanillin after extraction in ethanol from crude drugs, essences and homeopathic preparations available in local market after extraction in ethanol. The amount found was within the range 0.02-1.26, 0.01-1.05 and 0.26-1.39 % with relative standard deviation (RSD) 0.01-0.3, 0.01-0.05 and 0.01-0.05 % (n = 3), respectively.

Key Words: Vanillin, 1-Aminonaphthalene, Spectrophotometry.

INTRODUCTION

Vanilla (*Vanilla planifolia* Andrews) is used in large number of sweet dishes as a flavouring agent. Vanilla is also used in cookie recipes, cakes, sweets, continental puddings, gruels, milk based sweet drinks and dry pastries. The most important, almost proverbial, application is vanilla ice-cream. The largest part of vanilla-flavoured industrial products does not contain true vanilla, but much cheaper vanillin. Although the vanillin enhances the flavour of the food, but if the large amount is ingested or even inhaled, it may cause headache, nausea and vomiting and could affect liver and kidney functions[1]. Several analytical methods have been reported for the analysis of vanillin, based on spectrophotometric[2,3], FIA[4], flourimetric[5], ion selective electrodes[6], thin layer chromatography[7-17], GC[18,19], capillary electrophoresis[20] and polarographic[21] techniques. For spectrometric analysis, the determination is carried out using either the natural absorbance of vanillin at 280 nm or it is derivatized with a suitable reagent. The derivatization either increases the molar absorptivity

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or produces bathochromic shift, therefore, 1-aminonaphthalene is examined for the first time as a derivatizing reagent for spectrophotometric determination of vanillin in food.

EXPERIMENTAL

All the chemicals and reagents used were of analytical or pharmaceutical grades. The double distilled water used throughout the study was obtained from distillation plant. Pure vanillin, 1-aminonaphthalene and acetic acid were obtained from E. Merck, (Germany), sodium acetate from Fluka, (Switzerland) and ethanol from BDH, (UK) were used. Buffer solutions between pH 1-10 at unit internal were prepared from hydrochloric acid (1 M), potassium chloride (1 M), acetic acid (1 M), sodium acetate (1 M), sodium bicarbonate (1 M), sodium carbonate (saturated solution), ammonium chloride (1 M) and ammonia solution (1 M). The solution of 1-aminonaphthalene (2 % w/v) was prepared by dissolving 2 g of 1-aminonaphthalene in ethanol (100 mL). The spectrophotometric studies were carried out with a double beam spectrophotometer UV-1601 (Shimadzu Corporation, Japan) with dual silica 1 cm cuvettes.

Analytical procedure: The aqueous solution (1-5 mL) containing vanillin (50-250 µg/mL) was transferred to 10 mL calibrated stoppered volumetric flasks and were added 2 mL 1-aminonaphthalene (2 % in ethanol w/v), followed by acetate buffer pH 7 (1 mL). The contents were heated on water bath at 90 ºC for 15 min. The solutions were cooled at room temperature and the volumes were adjusted to mark with ethanol. The absorbance was measured at 372 nm against reagent blank which was prepared in a similar way only omitting the addition of vanillin.

Reference UV method: The solution (1-5 mL) containing vanillin (05-25 µg) was transferred to 10 mL calibrated stoppered volumetric flasks and the volumes were adjusted to mark with ethanol. The absorbance was measured at 280 nm against ethanol the molar absorptivity was observed 10800 L mol^{-1} cm^{-1}.

Analysis of vanillin from crude drugs: The sample (5 g) from each of the vanilla pod (Sri Lanka), Bijasil, Ood, Asafoetida, Clove and Benzoin (Pakistan) was crushed to coarse particles and macerated with ethanol (100 mL). The ethanolic extract was filtered twice using Whatmann filter paper No. 42. The ethanolic extract (1 mL) was transferred to volumetric flask (10 mL) and the procedure was repeated as described in analytical procedure.

Analysis of vanillin from homeopathic preparations: The sample (0.1 mL) from each of the Asafoetida mother tincture (Willmar Schwabe, Germany), Gastone syrup (Vital Homeopathic Pharma, Karachi, Pakistan) and Brooks combination number 3 (Brooks Homeotabs, Karachi, Pakistan), was taken in different volumetric flask (10 mL) and the procedure was repeated as described in analytical procedure.

Analysis of vanillin from essence samples: The samples (8.5 g) from sandal agarbatti (Metromillan Pvt. Ltd., Pakistan) and 2.5 g from Sandal tablets (Gulf Flavor Company, Dubai) were crushed to coarse particles and macerated in ethanol (100 mL) for 48 h. The solutions were filtered twice using Whatmann filter paper

No. 42. Finally, 1 mL from each sample was taken in separate calibrated volumetric flasks (10 mL) and the procedure was repeated as described in analytical procedure.

The sample (0.1 mL) from each sandal essence (Gulf Flavor Company, Dubai), clove essence, vanilla essence and attar (SMC Lahore, Pakistan), was taken in different volumetric flasks (10 mL) and the volume was adjusted to the mark with ethanol. The sample (1 mL) from diluted solution of sandal essence and attar and the sample 0.1 mL from clove essence and vanilla essence were taken in separate volumetric flasks (10 mL) and the procedure was repeated as described in analytical procedure. The amount of vanillin from each of the sample was calculated using the external calibration curve.

% Recovery of vanillin from samples by standard addition

Crude drugs: Vanilla pod (5 g) was dissolved in 100 mL ethanol, macerated for 24 h filtered and two portions of filtrate 0.2 mL each were taken. This was added with 2 mL of 100 µg/mL VAN solution and the derivatization procedure was followed for both solutions as described in analytical procedure.

Homeopathic preparation: Asafoetida mother tincture, (0.1 mL) was taken in duplicate and a solution was added 2 mL of 100 µg/mL vanillin standard solution and the derivatization procedure was followed for both solutions as described in analytical procedure.

Essence samples: The sandal agarbatti (5 g) was dissolved in 50 mL ethanol and allowed to stand for 24 h and filtered. The filtrate 1 mL was taken in duplicate and a solution was added 2 mL of 100 µg/mL vanillin standard solution and the derivatization procedure was followed for both solutions as described in analytical procedure. The % recoveries were calculated from the increase in the absorbance with added standards which were 98, 96 and 90 %, respectively.

RESULTS AND DISCUSSION

Vanillin reacts with 1-aminonaphthalene to form an azomethine derivative α-(vanillylidene)-aminonaphthalene (VAN-AN) (Fig. 1) which absorbs maximum with bathochromic shift to 372 nm with molar absorptivity of 5540 L mol^{-1} cm^{-1}. 1-Aminonaphthalene was then examined as a derivatizing reagent for the spectrophotometric determination of vanillin. The effects of pH, amount of 1-aminonaphthalene added, heating time and temperature on the formation of VAN-AN derivative were studied.

Optimization of parameters

Analytical wavelength: For the quantitative analysis, the wavelength of maximum absorbance plays an important role. It is necessary to check that derivatizing reagent should not absorb close to the region where the analyte derivative absorbs. This may cause error in absorption of the drug because the derivatizing reagent is added in excess to complete the reaction quantitatively. To avoid this problem, it is necessary to select the wavelength where the derivatizing reagent indicates minimum absorbance and the analyte derivative shows maximum absorbance value.

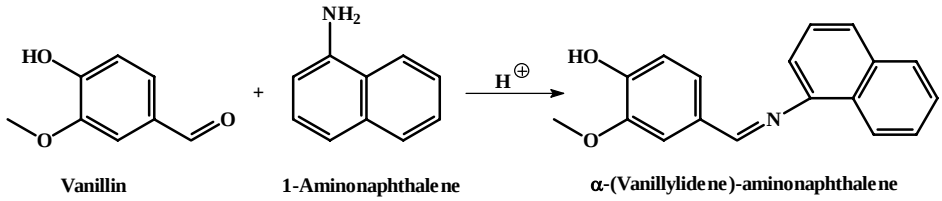

Fig. 1. Formation of derivative VAN-AN by reaction of VAN with AN

The absorbance value of 5 µg/mL of vanillin as 1-aminonaphthalene derivative was recorded at different wavelengths between 250-500 nm after heating for 15 min at 90 ºC using buffer pH 7. It is evident that the maximum absorbance occurs at 372 nm against reagent blank, therefore, the wavelength of 372 nm and was selected as optimum wavelength (λ_{max}).

Effect of reagent concentration: The effects of adding various amounts of 1-aminonaphthalene solution on absorbance of 5 µg/mL vanillin is given in Fig. 2. The concentration of reagent 1-aminonaphthalene was varied between 1-5 mL of 2 % in ethanol with an interval of 1 mL and the absorbance was measured at λ_{max} 372 nm. The similar absorbance was observed with addition of 1.5 mL and above and addition of 2 mL (2 % w/v) 1-aminonaphthalene solution and that was selected.

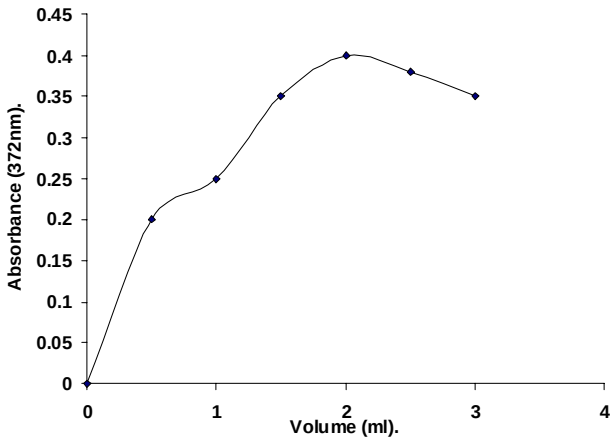

Fig. 2. Effect of reagent concentration on absorbance of vanillin derivative

Effect of order of mixing the reagents: The order of adding reagents during derivatization process has important role in accuracy of results and enhancement of absorbance.

In the present study, it was observed that the addition of buffer pH7 (1 mL) to 50 µg vanillin solution (1 mL) followed by the reagent (2 mL) resulted in a decrease in absorbance value. Taking the reagent first and then adding the buffer, followed

by vanillin solution also gave lower absorbance value. The maximum absorbance value was observed when 2 mL of reagent 1-aminonaphthalene was added to the standard solution of vanillin followed by buffer (1 mL) pH 7. The contents were then heated on water bath and the volume was adjusted to the mark with ethanol.

Optimization of heating time and temperature for the formation of derivative: To achieve the maximum absorbance value for an analyte, the selection of optimum time and temperature for the formation of stable derivative are essential parameters. The effect of time on absorbance of 05 µg/mL vanillin solution in the presence of 2 % 1-aminonaphthalene solution was checked at 372 nm from 0-30 min with an interval of 5 min. A similar absorbance was observed after heating for 15 min. Therefore, the heating time of 15 min at 90 ºC was considered as optimal.

Effects of solvents: The effect of various solvents such as methanol, 1-propanol, 1-butanol, amyl alcohol, isoamyl alcohol, acetonitrile, ethyl acetate, toluene, nitrobenzene and carbon tetrachloride on the absorbance of 10 µg/mL vanillin was examined. Each of the solvent 1 and 2 mL was added after the addition of 2 % ethanolic solution of 1-aminonaphthalene and 1 mL acetate buffer pH 7 followed by heating for 15 min. The ethanol proved to be the best choice while the addition of H_2O produced turbidity.

Effect of pH: The effect of adding 1 mL of 1 M buffers of pH range 1-10 on the absorbance of 5 µg/mL vanillin solution at already optimized conditions was studied.

It is evident from Fig. 3 that the absorbance increased gradually from buffer pH 1 and it was maximum at pH 7. Addition of buffer above pH 8 produced precipitation. Therefore, the acetate buffer of pH 7 was selected as optimal.

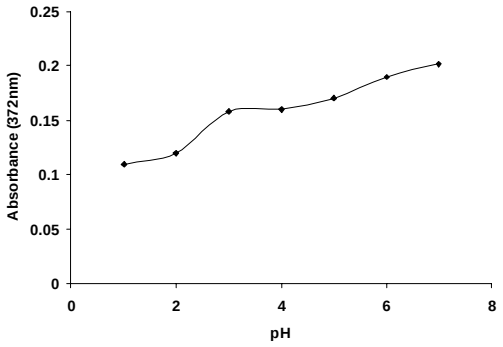

Fig. 3. Effect of pH on derivatization of vanillin

Interference study: The effect of possible presence of associated materials such as mannitol, sorbitol, lactose, sucrose, glucose, galactose and fructose was investigated at 10 times the concentration of vanillin and it was observed that none of these substances interfered with change in absorbance less than 4 %.

Stability of the derivative: The stability of VAN-AN derivative was examined in terms of absorbance at the concentration of 10 µg/mL VAN, but no change in absorbance of more than 5 % was observed within 48 h.

Calibration plots: The effect of variation in the concentration of vanillin on its absorbance as derivative VAN-AN was studied. A linear calibration curve was obtained which obeyed Beer's law within the concentration range 5-25 µg/mL of vanillin with coefficient of determination r^2 0.9995 (Fig. 4). The sandells sensitivity (0.004) was observed at 2 µg/mL VAN-AN. The validity of the calibration curve was obtained by the analysis of test solution of vanillin and the percent relative error was found ± 1-2 %.

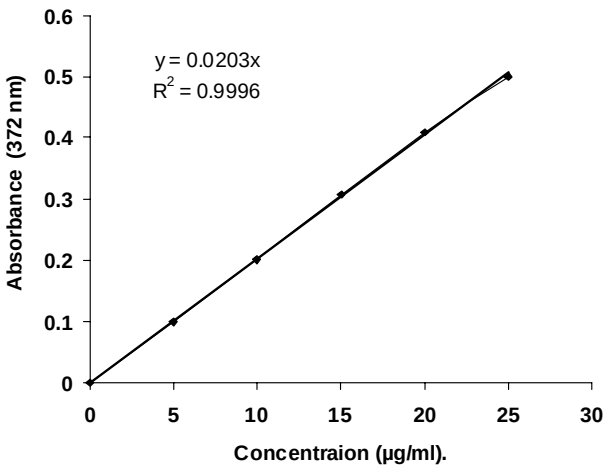

Fig. 4. Calibration curve of vanillin (0.005 %) using 1-aminonaphthalene as a derivatizing reagent

The samples containing vanillin available in the local market were analyzed to determine the amount of vanillin quantitatively. Vanilla pod, bijasil, ood, asafoetida, clove and benzoin were analyzed as crude drugs, sandal attar, sandal tablets, clove essence, vanilla essence and sandal agarbatti, were analyzed as essence samples and asafoetida (mother tincture), gastone syrup, brooks combination number 3 were analyzed as homeopathic preparations (Table-1).

The mean values and (95 % confidence limit) of 6 crude drugs, 5 essences and three homeopathic preparations were 0.403 ± 0.2107, 2.197 ± 3.65 and 0.774 ± 0.338, respectively (Table-2).

Day to day reproducibility/repeatability: For the determination of intra and interday reproducibility of the method, the ethanolic standard solution (2 mL) of 100 µg/mL vanillin was taken in three different calibrated volumetric flasks (10 mL) and the procedure was followed as described in analytical procedure. The absorbance was measured against reagent blank at 372 nm. The above procedure was repeated for 3 d (n = 3). The mean absorbances of intraday and interday reproducibilities were observed as 0.204 and 0.206 with (RSD) values 0.036 and 0.0015 %, respectively.

TABLE-1
ANALYSIS OF VAN FROM CRUDE DRUGS, HOMEOPATHIC PREPARATIONS AND SYNTHETIC ESSENCES

Name of Crude drug	Amount found % (standard deviation)
Vanilla pod	1.26 (0.01)
Bijasil	0.36 (0.02)
Ood (Eagle wood)	0.16 (0.01)
Asafoetida	0.39 (0.03)
Clove	0.20 (0.02)
Benzoin	0.02 (0.02)
Asafoetida (mother tincture)	1.39 (0.02)
Gastone syrup	0.66 (0.15)
Sandal tablets	0.02 (0.01)
Sandal Agarbati	0.01 (0.01)
Sandal (Attar)	0.16 (0.01)
Clove essence	0.13 (0.01)
Vanilla essence	1.05 (0.90)

TABLE-2
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY

Parameter(s)	Values
Beer's law limits (μg mL^{-1})	05-25
Molar absorptivity (L mol^{-1} cm^{-1})	5540
Sandells sensitivity (μg/mL/0.004 absorbance unit)	2
Regression equation (y)[a]	
Slope (b)	0.0203
Intercept (a)	0
Coefficient of determination	0.9995
Relative standard deviation	0.0015-0.152
Mean value of six crude drugs (samples) ± (95 % confidence limit)	0.403±0.2107
Mean value of five essences (samples) ± (95 % confidence limit)	2.197±3.65
Mean value of three homeopathic samples ± (95 % confidence limit)	0.774±0.338

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