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A Novel Spectrophotometric Method for Determination of Five 1,4-Dihydropyridine Drugs in Their Tablets and Capsules Using Vanillin Reagent

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ABSTRACT

A selective and new spectrophotometric method is described for determination of five 1,4-dihydropyridine drugs (1,4-DHP); namely nifedipine (NIF), nicardipine (NIC), nimodipine (NIM), felodipine (FEL) and amlodipine (AML). The method is based on a coupling reaction between the cited drugs and vanillin reagent in acidic condition. Under optimized conditions, the red coloured products were measured at 500 nm for NIF, NIC, NIM and FEL or at 479 nm for AML. Molar absorptivities were ranged from 0.575×10^4 - 1.065×10^4 $l \cdot mol^{-1} \cdot cm^{-1}$, Beer's law was obeyed at 5 - 70 µg/mL concentration range and the limit of detection was ranged from 0.150 - 1.500 µg/mL. The proposed method was successfully extended to pharmaceutical preparations tablets and capsules and comparison by Student's t-test and variance ratio F-test showed no significant difference.

Keywords: Spectrophotometric Method; 1,4-DHP; Validated; Selective; Vanillin Reagent

1. Introduction

1,4-DHP drugs, as shown in **Figure 1**, are primarily used for treatment of cardiovascular diseases such as hypertension, angina and some forms of cardiac arrhythmias. These agents are useful in other pathological states, such as seizures and central ischemic disorders through their action on slow L-type channels [1,2] also they have the advantages of little interaction with other cardiovascular drugs, such as digoxin or warfarin that are often used concomitantly with them [3]. The therapeutic importance and successful clinical uses of these drugs have promoted the development of many analytical methods for their determination in bulk, in their pharmaceutical formulations and in biological fluids. Analytical techniques such as; titrimetric methods [4,5], spectrometric methods (spectrophotometry [6-13] or spectrofluorimetry [13-20]), electrochemical methods [21-23], liquid chromatographic methods [24-28] and gas chromatographic methods [29-32] were reported for their determination.

The inherent simplicity of spectrophotometric methods, economic advantages and availability of their instruments in most quality control laboratories permit development of a simple and selective method for determine these drugs. The proposed method involved treatment of the investigated drugs directly with vanillin reagent (**Figure 2**); in the presence of HCl acid to give coloured products measured at specific wavelengths.

2. Experimental

2.1. Instruments

Absorbance measurements were made on Shimadzu model 1601PC, UV-Visible Spectrophotometer (Shimadzu, Tokyo, Japan) and Jenway 6305, UV-Visible Spectrophotometer, UK (Jenway LTD).

2.2. Chemicals

All chemicals, solvents and reagents were spectroscopic grade and their solutions were prepared in distilled water.

2.2.1. Vanillin Reagent

Vanillin (El Gomhouria Co, Cairo, Egypt) concentration was 0.5%, w/v for NIF, NIC, NIM and FEL. Prepared by dissolving 500 mg of vanillin in 2.0 mL methanol then

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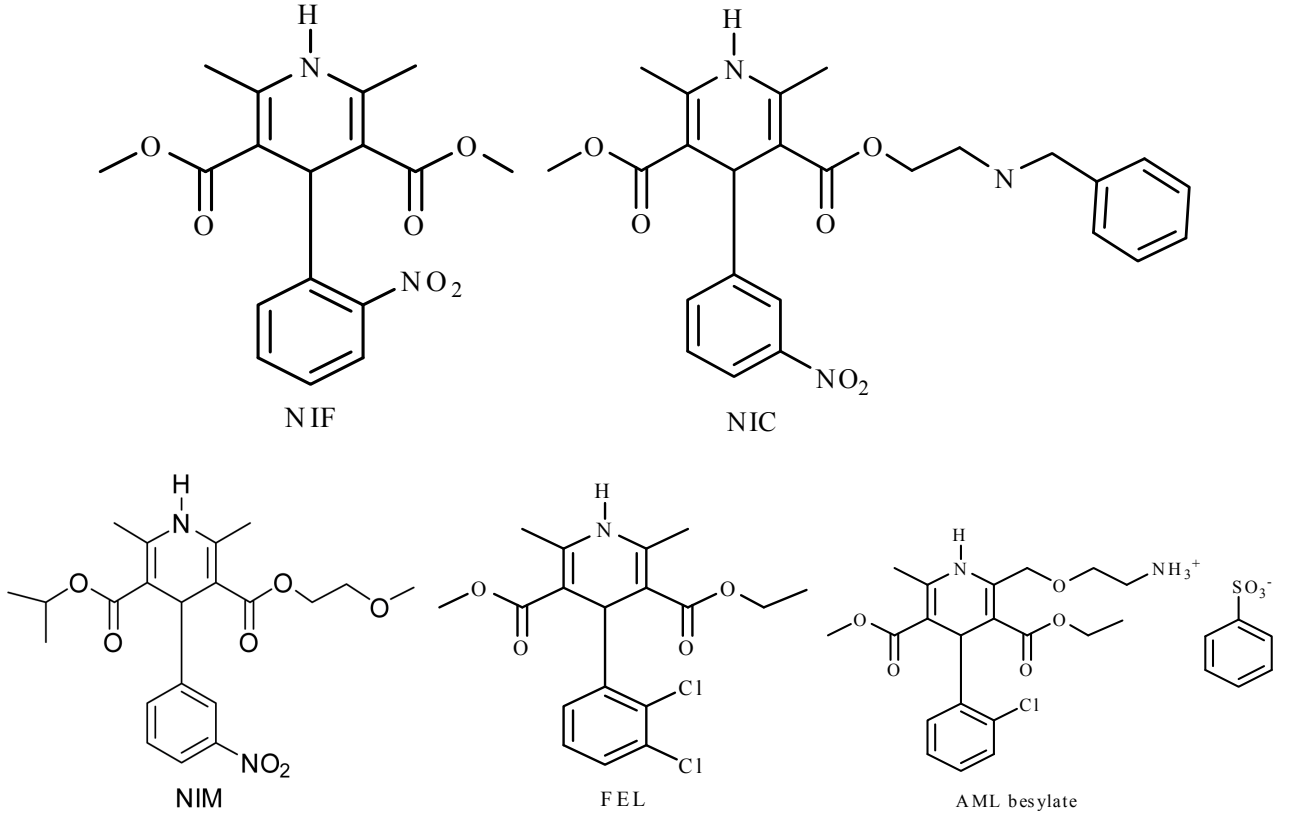


Figure 1. Chemical structures of the investigated 1,4-DHP drugs.

diluted to 100 mL with HCl (35.5%, w/v).

Vanillin concentration was 2.0% w/v for AML, prepared by the same steps in the previous method.

2.2.2. 1,4-DHP Drugs Stock Solution

Reference standards of purecited drugs (NIF, atenolol, NIC HCl, NIM, FEL, AML besylate and Metoprolol) were generously supplied by their respective munufacturers.

A standared solutions were prepared by dissolving 50 mg of NIF, NIC and FEL and 100 mg for NIM and AML in 2.0 mL methanol then the resulting solution were completed to 100 mL by HCl (35.5% v/v), the working standard solutions were prepared by further dilution with aqueous HCl (17.75%, v/v) to obtain a concentration range of 5.0 - 80 µg/mL.

Stock standard of 1,4-DHP solutions was freshly prepared and kept in dark containers due to their photosensitivity [33].

2.3. Procedure for Calibration Curves

An aliquot of 1.0 mL of the standard drugs solution was transferred into a 10-mL calibrated flask. 1.0 mL of vanillin reagent was added, mixed well, the reaction was allowed to proceed for 30 min at 50˚C, for NIF, NIC, NIM and FEL or 35 min at 70˚C, for AML then the resulting coloured products were measured at 500 nm for NIF, NIC, NIM, FEL or 479 nm for AML; against blanks which treated similarly.

2.4. Procedure for the Assay of Tablets and Capsules

Twenty tablets or capsules were weighted accuratly, the contents were mixed thoroughly and a quantity of the powder equivalent to 12.5 mg of the active ingredients of NIF, NIC and FEL or 25 mg of NIM and AML was dissolved in 2.0 mL methanol. The contents were swirled and sonicated for 5 min, then filterted through a Whatmann No. 42 filter paper previously moisted with methanol. The collected filtrate was transferred quantitatively into 25-mL calibrated flask, the resultant solution was completed to mark with HCl (35.5% v/v) and then subjected to subsequent dilution.

2.5. Procedure for the Assay of Tablets and Capsule Containing Two Drugs

Twenty tablets (Logimax® tablets) or capsules (Tenolat SR® capsules) were weighted and finely powdered, then

complete as the same procedure as montionedin 2.4.1.

3. Results and Discussion

Adding vanillin reagent to 1,4-DHP drugs in the presence of hydrochloric acid and at a high temperature, showed a red colour which can be measured spectrophotometrically (**Figure 3**), this is a novel reaction for detremination of these drugs and it was suggested as a coupling reaction between the aldehydic group of vanillin and active methyl group which present in all the cited drugs, the suggested mechanism involved one reaction step and did not depend on the presence of the -NO_3 group as in some cited drugs structure (NIF, NIC and NIM). In reported method [34] this -NO_3 group was needed reduction firstly and then coupled with reagent contains aldehydic group.

3.1. Optimization of Parameters

A series of experiments were conducted to establish the optimum experimental conditions for the proposed method.

3.1.1. Reagent Concentration

Product's colour development was dependant on vanillin reagent concentration and the absorbance values were increased as the concentration of vanillin increased. So, 7.5 mg/mL of vanillin was selected for NIF, NIC, NIM and FEL experiments (**Figure 4**) or 20 mg/mL of it in case of AML experiment (**Figure 5**).

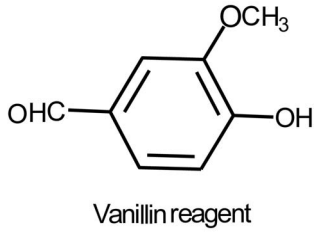


Figure 2. Chemical structures of vanillin reagent.

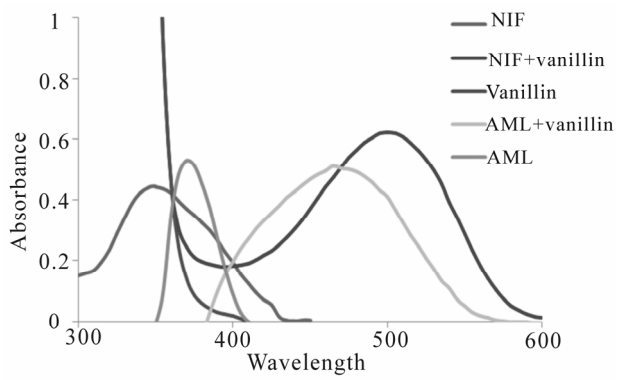


Figure 3. Absorption spectra of NIF (20 µg/mL), NIF-vanillin, vanillin reagent, AML-vanillin and AML (50 µg/mL).

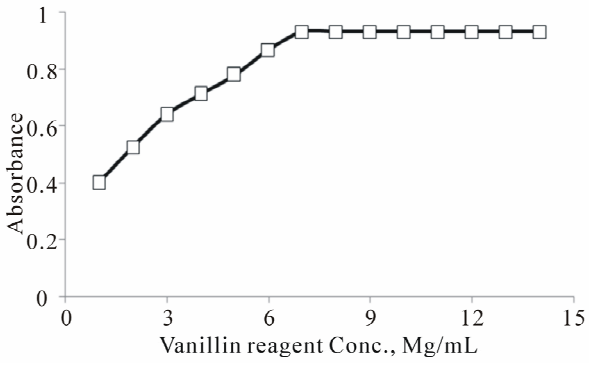


Figure 4. Effect of vanillin concentration on the absorbance intensity of NIF-Vanillin reaction product (NIF Conc. 25 µg/mL).

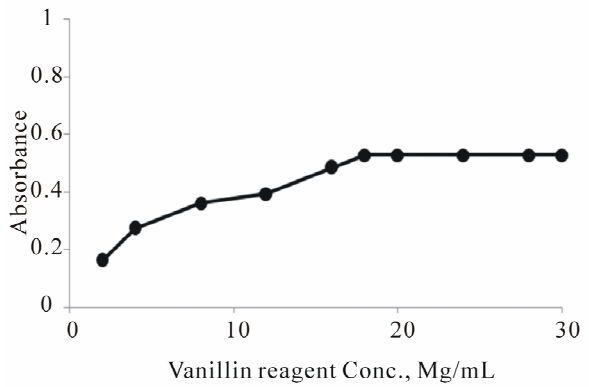


Figure 5. Effect of vanillin concentration on the absorbance intensity of AML-vanillin reaction product (AML Conc. 50 µg/mL).

3.1.2. Acid Type and Its Concentration

Reaction between vanillin reagent and 1,4-DHP drugs was found to proceed in acidic medium. So, different acids were tested **Table 1**. Hydrochloric acid resulted in an increase of the absorbance intenisty accompained by hyperchromic shift.

3.1.3. Temperature and Reaction Time

The effect of temperature was studied in the range of 25˚C - 100˚C for different periods of time (5 - 35 min). The reaction product's absorbance was increased by increasing the temperature up to 50˚C for 30 min, in case of NIF, NIC, NIM and FEL and the reaction product's colour was stable after this time and more than 40 min (**Figure 6**), NIF as representative example as shown in **Scheme 1**.

For AML, increasing temperature up to 70˚C for 35 min increased the absorbance intensity and the reaction product's colour was stable more than 40 min (**Figure 7**).

3.2. Reaction Stoichiometry

Job's method of continuous variation [34] was employed

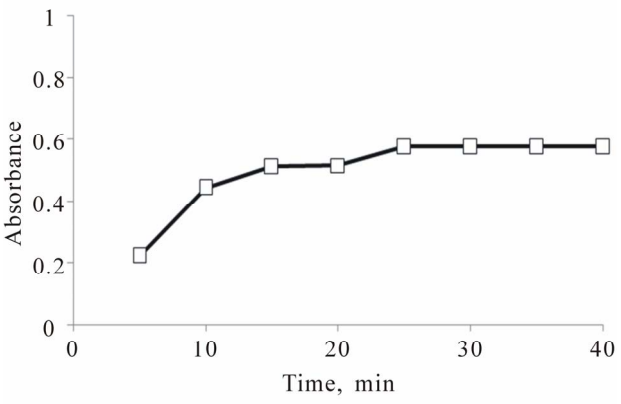


Figure 6. Effect of time on NIF (20 µg/mL) with vanillin (7.5 mg/mL) at 50˚C.

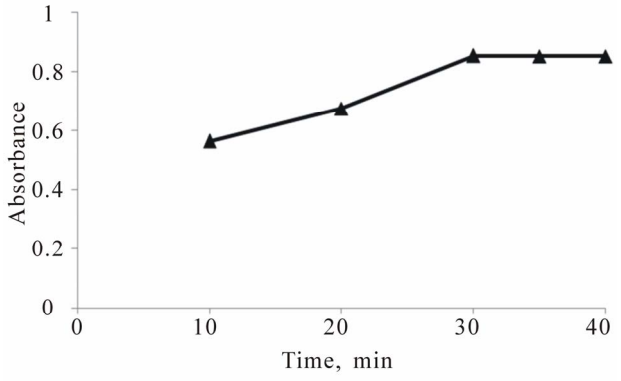


Figure 7. Effect of time on AML (50 µg/mL) with of vanillin (20 mg/mL) at 50˚C.

to establish the stoichiometry of the reaction; Mastere quimolar solutions 5×10^{-3} M of vanillin reagent, NIF, NIC, NIM and FEL or 1.5×10^{-2} M of both vanillin reagent and the AML were prepared. Series of 10-mL portions of the master solutions were made up comprising different complimentary proportions (0.00:0.10, 0.10: 0.90, …, 0.90:0.10, 0.10:0.00) in 10-mLvolumetric flasks, mixed well then subjected to the recommended procedure. The stoichiometry of the reaction between the investigated drugs and vanillin, revealed a 1:1 for all drugs (**Figure 8**).

Suggested Reaction Mechanism between 1,4-DHP and Vanillin Reagent

The reaction mechanism is under investigation. As reported reaction between active methyl group and formaldehyde [35]; the reaction between the active methyl group and aldehydic group of vanillin reagent was proceed via nuclophilic addition of double bond to vanillin to form a carbonium ion which undergoes deprotonation followed by lossing molecule of water under acidic conditions, to yield the conjugated coloured product, as shown in **Scheme 1**.

3.3. Validation of Proposed Methods

The developed procedures were fully validated according to USP XXVI [36] validation guidelines and International Conference on Harmonization (ICH) [37] guidelines.

3.3.1. Linearity Range, Detection and Quantification Limits

Under the specified optimum reaction conditions, the calibration curves for the investigated drugs; NIF, NIC, NIM, FEL, and AML with vanillin were constructed by analyzing a six or seven concentrations of the drugs standard (**Figure 9**). Good linearity between different drugs concentration and the practical absorptions, is indicated by the high correlation coefficients (*r*) (0.9987 - 0.9999). The regression equations for the results were derived using the least square method: A = a + bC.

The limits of detection (LOD) and limits of quantitation (LOQ) were determined according to the IUPAC definitions [38] using the formula: LOD or LOQ = κSDa/b; where κ = 3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope. The obtained results were summarized in **Table 2**.

3.3.2. Precision

The precision was determined by carrying out the replicate analysis of five separate standard at one concentration level of each drug according to USP XXV validation guidelines [39]. The relative standard deviations did not exceed 2% indicating the good repeatability of the proposed method (**Table 3**).

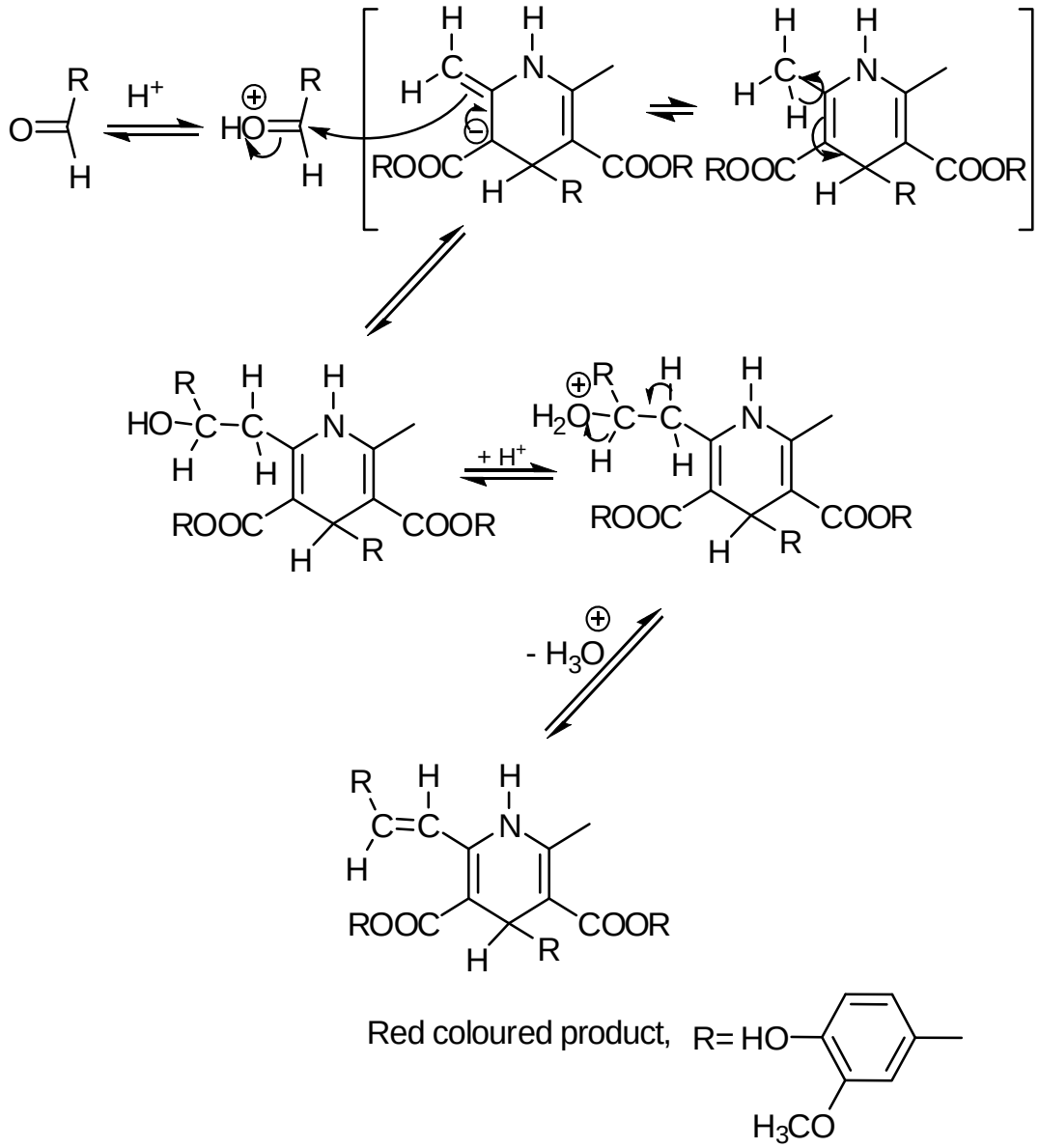
The intra-day precision was assessed by analyzing three replicates of each sample as a batch in a single assay run, and the inter-day precision was assessed by analyzing the same sample, as triplicate, in two separate runs. The relative standard deviations did not exceed 2% indicating the good reproducibility of the proposed method.

3.3.3. Selectivity

The selectivity of the proposed method was checked by monitoring the drugs standard solutions in the presence of other ingredients which present in their tablets and capsules [40]. The results we obtained revealed that the response was not significant different from that results that we obtained in case of pure drugs in calibration curve (**Table 4**) using NIF as representative example.

3.3.4. Robustness and Ruggedness

Method robustness was examined by evaluating the influence of small variation in some experimental parameters such as the concentration of analytical reagent and reaction times on the method's suitability and sensitivity.



Scheme 1. Suggested reaction mechanism between 1,4-DHP drugs and vanillin reagent.

Table 1. Effect of acids type on the absorption intensity of the drugs-vanillin reaction products.

Acid[a]	Wavelength (nm)	Absorbance				
		NIF[b] 20 µg/mL	NIC[b] 20 µg/mL	NIM[b] 40 µg/mL	FEL[b] 20 µg/mL	AML[c] 50 µg/mL
Acetic	-	-	-	-	-	-
Hydrochloric	500	0.635	0.534	0.559	0.555	0.526
Nitric	463	0.136	0.123	0.198	0.131	0.111
Perchloric	-	-	-	-	-	-
Sulphuric	437.5	0.100	0.078	0.112	0.089	0.122

[a]11.5 M of acid conc. [b]7.5 mg/mL of vanillin. [c]20 mg/mL vanillin. -No Results.

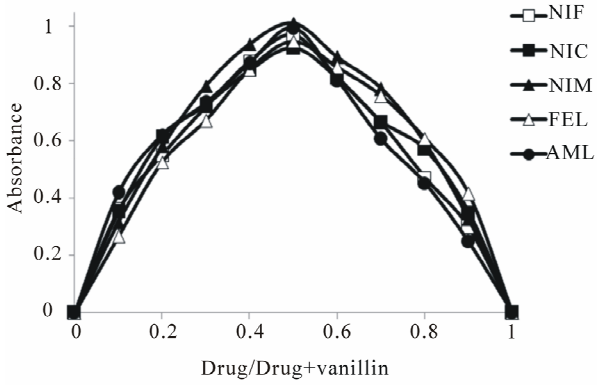


Figure 8. Job's plot for reaction of 5×10^{-3} M of vanillin-NIF, -NIC, -NIM and -FEL or 1.5×10^{-2} M of vanillin-AML.

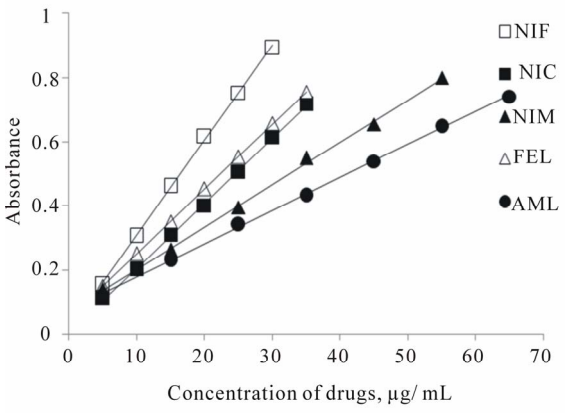


Figure 9. Calibration curves obtained from the reactions of standard drugs with vanillin reagent.

In these experiments, one parameter was changed where as the others were kept unchanged and the recovery percentages were calculated at each time. It was found that none of these variables significantly affect the proposed methods **Tables 5** and **6**.

3.3.5. Acuracy and Applications

A good satisfactory results which obtained by the proposed method for the investigated drugs in bulk forms also were extended to cited drugs analysis in their tablets and capsules forms **Table 7**.

The results were compared with those obtained by the official [33] and other reported methods [41,42] with respect to the accuracy (t-test) and precision (F-test). No significant differences were found between the calculated and theoretical values of both the proposed and the official or reported methods at 95% confidence level which indicated similar accuracy.

4. Conclusion

The colour formed under the above mentioned conditions can be regarded as a coupling reaction between the aldehydic reagent and active methyl group. Compared with other reported methods, the proposed methods have the advantages of simplicity, sensitivity, selectivity and reproducibility and it satisfies the need for a rapid procedure for the determination of all members of 1,4-DHP drugs which containing active methyl group using different reagents contain aldehydic groups.

Table 2. Quantitative parameters and statistical data for the studied drugs with vanillin reagent.

Drug	Linear range (µg/mL)	Intercept[a] ± SD, (N = 5)	Slope ± SD, (N = 5)	Corr. Coeff. (r)	$\varepsilon \times 10^4$ ($l \cdot mol^{-1} \cdot cm^{-1}$)	LOD (µg/mL)	LOQ (µg/mL)
NIF	5.00 - 30.0	0.013 ± 0.007	0.029 ± 0.0003	0.9993	1.065	0.724	2.413
NIC	5.00 - 35.0	0.003 ± 0.005	0.020 ± 0.0002	0.9994	1.042	0.750	2.500
NIM	10.0 - 60.0	0.003 ± 0.009	0.013 ± 0.0002	0.9987	0.572	2.076	6.923
FEL	5.00 - 35.0	0.049 ± 0.001	0.020 ± 0.0001	0.9999	0.846	0.150	0.500
AML	10.0 - 70.0	0.024 ± 0.005	0.010 ± 0.0001	0.9994	0.610	1.500	5.00

[a]n = five determinations.

Table 3. Assay of five replicate samples of the studied drugs by vanillin reagent at one-concentration level.

Drug	Conc. (µg/mL)	Absorbance difference					Mean ± SD	RSD
		1	2	3	4	5		
NIF	25.00	0.610	0.592	0.615	0.601	0.605	0.605 ± 0.0078	1.301
NIC	25.00	0.500	0.513	0.510	0.513	0.500	0.507 ± 0.0059	1.179
NIM	40.00	0.547	0.543	0.541	0.548	0.549	0.546 ± 0.0031	0.563
FEL	25.00	0.531	0.532	0.529	0.527	0.531	0.531 ± 0.0018	0.339
AML	50.00	0.538	0.533	0.537	0.529	0.530	0.533 ± 0.0036	0.677

Table 4. Effect of interference on the determination of the studied drugs by vanillin reagent.

Interference	Amount added, mg	Recovery (% ± SD)[a]
Excipients[b]		
Starch	(50)	98.51 ± 0.91
Sucrose	(50)	97.58 ± 0.74
Lactose	(10)	99.05 ± 1.67
Glucose	(10)	97.33 ± 0.57
Mg stearate	(5)	96.51 ± 0.91
Talc	(5)	98.63 ± 0.27
Combined drugs		
Atenolol	(50)	98.24 ± 0.67
Metoprolol[c]	(50)	99.44 ± 0.46

[a]N = 5. [b]The amount of excipients added/20 mg of NIF as representative example. [c]metoprolol plus 5 mg FEL.

Table 5. Robustness of the proposed method for determination of NIF, NIC, NIM and FEL.

Variation	Recovery % ± SD[a]			
	NIF 20 µg/mL	NIC 20 µg/mL	NIM 40 µg/mL	FEL 20 µg/mL
No variation	99.17 ± 0.64	99.10 ± 0.61	99.23 ± 1.45	99.03 ± 0.85
Vanillin concentration				
7 mg/mL	98.98 ± 0.71	99.07 ± 0.89	97.67 ± 1.89	98.78 ± 0.97
8 mg/mL	98.94 ± 0.72	99.01 ± 0.62	98.71 ± 1.22	98.70 ± 0.91
Reaction time				
28 min	98.93 ± 0.81	99.10 ± 0.40	98.46 ± 0.89	98.60 ± 0.92
32 min	99.01 ± 0.59	98.86 ± 0.88	97.61 ± 0.62	98.80 ± 0.90

[a]N = 3.

Table 6. Robustness of the vanillin method for analysis of the AML drug.

Variation	Recovery % ± SD[a]
	AML, 50 µg/mL
No variation	99.90 ± 0.90
Vanillin concentration	
9 mg/mL	97.50 ± 0.87
21 mg/mL	98.80 ± 0.76
Reaction time	
33 min	97.70 ± 0.98
37 min	99.30 ± 1.10

[a]N = 3.

Table 7. Determination of the studied drugs in their tablets and capsules using vanillin reagent method.

Product	Recovery % ± SD[a]		F-value[b]	t-value[b]
	Proposed method	Official or reported method		
Epilate® capsules	99.78 ± 0.81	99.52 ± 0.11	1.53	1.64
Epilate Retard® tablets	98.76 ± 1.04	98.51 ± 0.13	2.52	1.44
Tenolat SR® capsules*	98.76 ± 0.72	100.52 ± 0.66	2.20	1.06
(Pelcard SR® capsules)[c]	99.00 ± 0.47	99.74 ± 0.11	2.57	1.84
Nimotop® tablets	98.32 ± 1.36	98.61 ± 0.19	2.21	1.35
Plendil® tablets	99.70 ± 0.89	99.24 ± 0.13	1.94	1.66
Plentopine® tablets	98.92 ± 0.62	99.22 ± 0.11	1.13	1.17
Logimax® tablets*	98.75 ± 0.78	99.34 ± 0.17	2.03	1.44
(Alkapress® tablets)[c]	97.75 ± 0.34	99.21 ± 0.12	2.50	1.41
(Myodura® tablets)[c]	99.76 ± 0.84	98.31 ± 0.11	1.74	1.82
(Amlodipine® tablets)[c]	98.58 ± 0.55	97.20 ± 0.12	3.20	1.06
(Regcor® tablets)[c]	98.00 ± 0.63	99.24 ± 0.16	2.57	1.84
(Vasonorm® tablets)[c]	99.06 ± 0.43	98.41 ± 0.19	1.94	1.66

[a]N= 5. [b]Theoretical values for F and t at 95% confidence limit (n = 5) were 6.39 and 2.78 respectively. [c]Reported methods [41,42]. *Combined drugs.

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