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Colorimetric method for determination of some 1,4-dihydropyridine drugs in their tablets and capsules

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ABSTRACT

A simple, accurate and selective colorimetric method was developed and validated for determination of five of 1,4-dihydropyridine drugs (1,4-DHP) using tetrabutylammonium hydroxide reagent (TBAH). The proposed method was based on addition of TBAH to the studied drugs then the produced yellow colors were measured spectrophotometrically. Different variables which affecting the reaction conditions were carefully studied and optimized. Under the optimum conditions, Beer's law was obeyed in the concentration range of 2.50-40.0 µg/mL and the limits of detection were ranged from 0.750-1.956 µg/mL. The proposed method was successfully extended to the pharmaceutical preparations, tablets and capsules. The obtained results were comparable with that obtained by the reference methods.

Keywords: Colorimetric method; five 1,4-DHP drugs; TBAH

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INTRODUCTION

Hypertension or high blood pressure is a cardiac chronic condition in which the systemic arterial blood pressure is elevated. It is classified either a primary (essential) hypertension or secondary hypertension. About 90-95% of cases are categorized as primary hypertensive and the remaining 5-10% of cases are secondary hypertensive patients. Although the dietary and lifestyle changes can improve the blood pressure and decrease the risk of associated health complications, but drug treatment may be necessary in patient for whom lifestyle changes are ineffective or insufficient.

Calcium ions play an important role in function of cardiovascular system and the calcium channel blockers (CCB) primarily exert their activities through inhibiting the calcium ion entry into the cells. Also CCB affect the cardiac and vascular smooth muscle cells and cause vasodilatation for both coronary and peripheral arteries, hence reducing the blood pressure [1, 2]. Nifedipine (NIF), nicardipine (NIC), nimodipine (NIM), felodipine (FEL) and amlodipine (AML), **Figure 1** are the prototypical 1,4-DHP derivatives and they are considered as CCB agents. Furthermore, they are useful in other pathological states, such as seizures and central ischemic disorders [3].

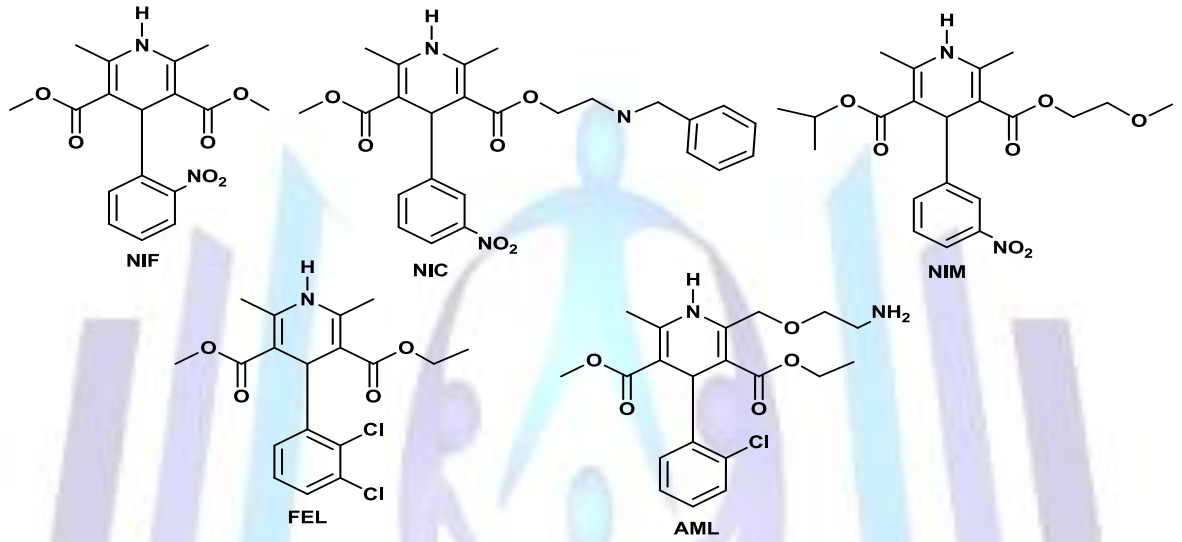

Fig 1.Chemical structures of the investigated 1,4-DHP drugs

Detailed survey of literatures for NIF revealed that several UV-Visible spectrophotometry methods that have been reported for its assay either alone or in combination with other drugs [4-13]. But only a few spectrophotometric and spectrofluorimetric methods have been reported for determination of NIC [10, 11,13-17]. NIM has another advantage than other its members, it is recommended for improvement of neurological outcomes by reducing the incidence and severity of ischemic deficits in patients with subarachnoid hemorrhage [18, 19]; for its assay a different spectrophotometric methods have been described in the present literatures [10, 11, 19-25]. In the quantitative determination of FEL in pharmaceutical dosage forms, spectrometric methods have been published [10, 11,26-29]. Also AML has been assayed in its pure and pharmaceutical dosage forms using different spectrometric methods [10, 11, 16, 30-51].

The main goal of this work is to establish a simple, accurate, precise, and reproducible spectrophotometric method for determination of these drugs in bulk and in their pharmaceutical preparations. Further, the established method should be rapid and economic to be applied for the routine quality control analysis or in pharmaceutical dosage forms.

EXPERIMENTAL

Instrumentation:

Absorbance measurements were made on Shimadzu-1601UV-Visible Spectrophotometer (Shimadzu, Tokyo, Japan), Jenway-6305 UV-Visible Spectrophotometer (Jenway LTD, U.K).

Reagents and solutions:

A 25 mg/mL of TBAH (Sigma Chemical Co., St. Louis, USA) solution was prepared in dimethylsulphoxide (DMSO) (Laba Chemie, Mumbai, India). Reference standards of pure drugs (NIF, NIC, NIM, FEL, AML and other combined drugs such as atenolol and metoprolol) were generously supplied from their respective manufacturers. 0.5 mg/mL of stock standard were prepared in DMSO; the working standard solutions were prepared by further dilution with DMSO.

Procedure for calibration curves:

An aliquot of 1.0 mL of the standard or sample solution was transferred into 10 mL calibrated flask. 1.0 mL of TBAH was added; the reaction was allowed to proceed for 20 min then diluted to the mark with DMSO. The absorbance of the resulting yellow color solutions were measured at 432, 447, 458, 457 and 464 nm for NIF, NIC, NIM, FEL, and AML respectively, versus experimental blank treated similarly.

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Pretreatment procedure for the assay of tablets and capsules:

The contents of twenty tablets or capsules were mixed thoroughly and quantities of the powder equivalent to 50 mg of the active ingredient were dissolved in 50 mL DMSO. The contents were swirled and sonicated for 5 min then the mixture was filtered. The collected filtrate was transferred into 100 mL calibrated flask and completed to the mark with DMSO.

Pretreatment procedure for the assay of tablet and capsule containing two drugs:

Twenty tablets (Logimax® tablets, FEL and metoprolol) or capsules (Tenolat SR® capsules, NIF and atenolol) were weighted accurately and the contents were mixed thoroughly. Quantities of the powder equivalent to 50 mg of the active ingredient of NIF and FEL were dissolved in 50 mL DMSO then the procedure was completed as the previous.

RESULTS AND DISCUSSION

The proposed method was focused on treatment of 1,4-DHP drugs with TBAH base in DMSO. **Figure 2** (AML, as a representative example) illustrates the bathochromic shift of the colored chromogen from the original spectrum of AML. Through the literatures; the corresponding anions of dihydropyridine are prepared by reaction of Hantzsch type dihydropyridines moiety with a strong base in a non-polar solvent, but unfortunately, nothing is known about the site of protonation, i.e. those have electron withdrawing substituents in both 3 and 5 positions [52, 53]. Exploratory experiments for such compound showed that the nature of the protonated species depended upon both the solvent and the base strength [54]. J. Luis *et al.* [55] explained the addition of TBAH base to 1,4-DHP drugs in DMSO produces a drastic bathochromic shift that is due to anion formation in the basic medium utilizing the proton in 1,4-DHP ring and the band gradually disappeared after addition of perchloric acid solution. Subsequently, as **Figure 3** shows, we verified this suggested neutralization reaction after color formation.

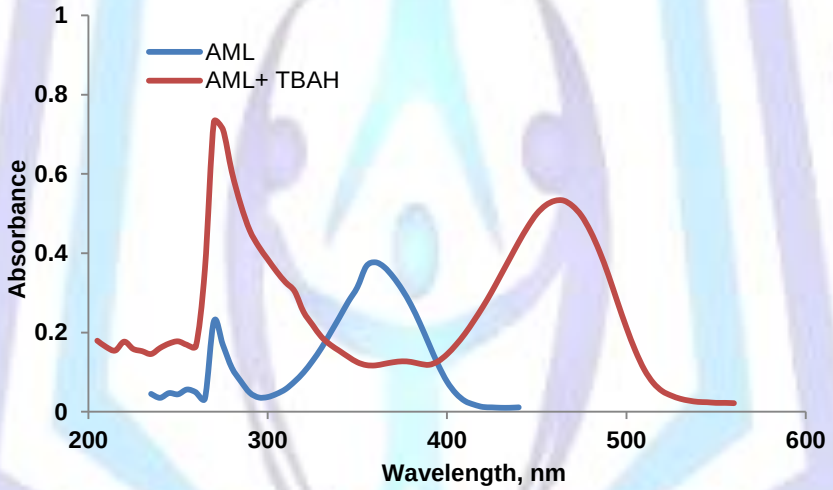

Fig 2. Absorption spectra of AML, 20 µg/mL and its colored product after addition of TBAH

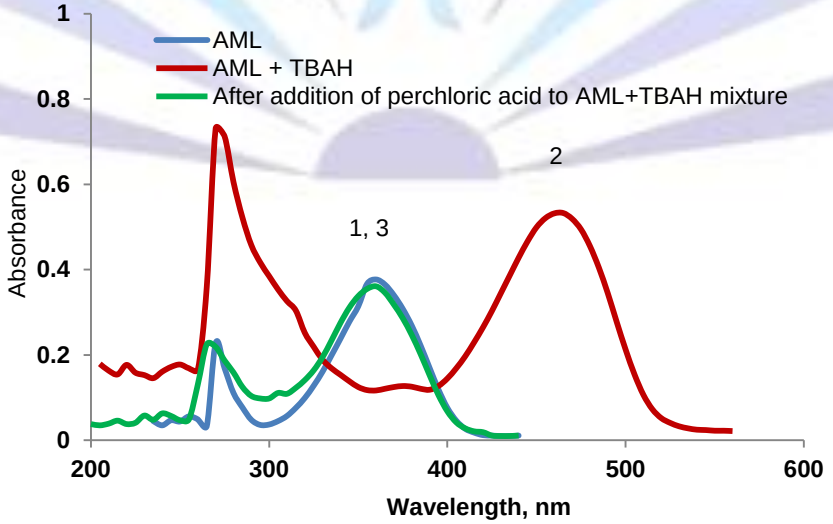

Fig 3. Absorption spectra of (1) AML, 20 µg/mL (2) AML after addition of TBAH (3) AML and TBAH reaction product after addition of 0.1 M perchloric acid, 1.0 mL

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OPTIMIZATION OF PARAMETERS:

Different variables such as type and concentration of the alkali, reaction time, temperature, and the diluting solvent, which influencing the intensity of the colored products were studied and optimized.

Basic reagent and organic solvents system:

Preliminary experiments were carried out to select the type of alkali and the organic medium; the results that were obtained revealed that the highest reaction products chromogen achieved when TBAH used compare to other used alkali such as NaOH, KOH and tetraethylammonum hydroxide solution. The greatest colors were obtained in dipolar aprotic solvents such as acetone, dimethyl formamide (DMF) or DMSO and the formed colors intensity increased relatively by increasing in dielectric constant of these solvents (20.7, 36.71 and 46.68 for acetone, DMF and DMSO, respectively [56]). Water and other protic solvent such as ethanol, methanol and propanol have a destructive effect on the formed chromogen. Therefore the highest and the stable absorption intensity for the produced colored products were observed when TBAH/DMSO system was used.

TBAH concentration:

25 mg/mL TBAH regent was selected from different studied concentrations ranged from 10-45 mg/mL of TBAH, **Figure 4**.

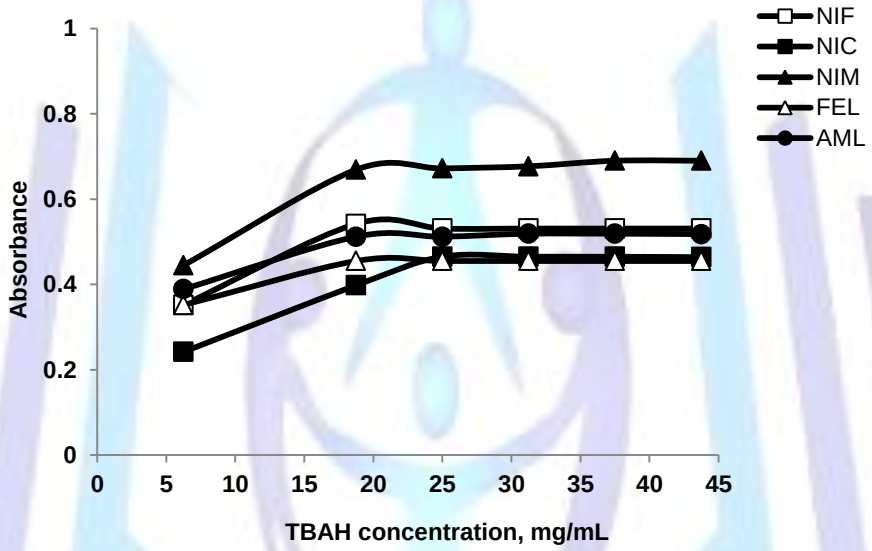

Fig 4. Effect of TBAH concentration on the absorption intensity of the reaction products with 1,4-DHPs, 20 µg/mL

Reaction time:

The reaction between drugs and TBAH was completed after 20 min at room temperature (25±5 °C) and the absorbance values of the colored products remained stable for more than 30 min after their dilution, **Figure 5**.

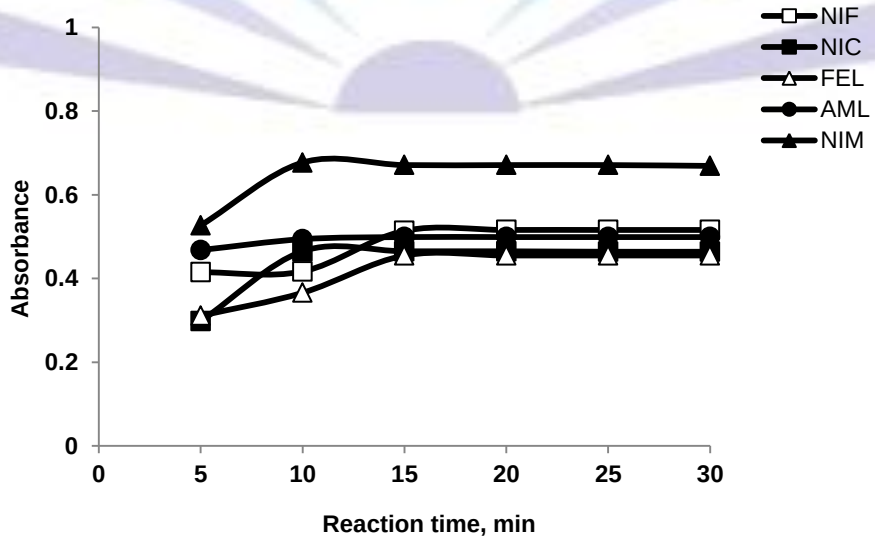

Fig 5.Time effect on the color intensity of the reaction products of TBAH with 1,4-DHP, 20 µg/mL

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REACTION STOICHIOMETRY

Job's method of continuous variation [57] was employed to establish the stoichiometry of proposed method. The ratios between the investigated drugs and TBAH were 1:2; **Figure 6** using NIF as representative example.

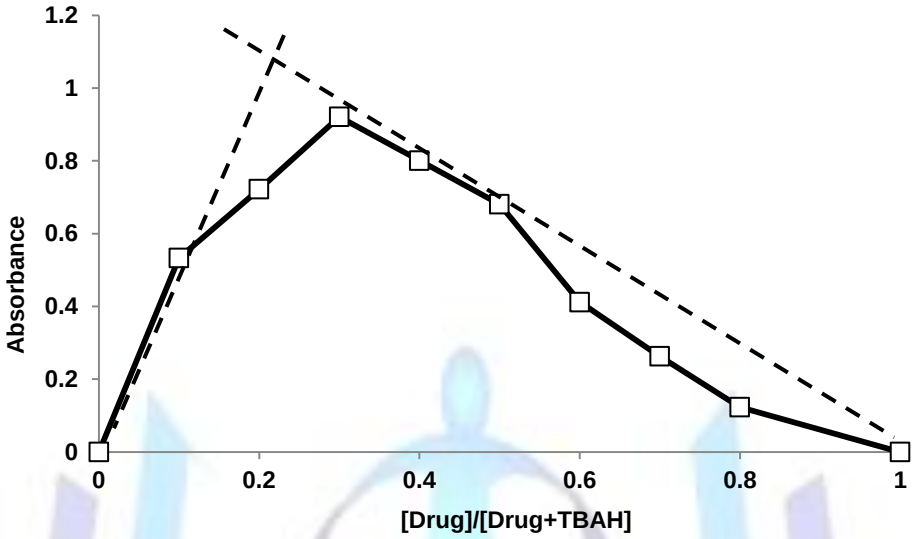
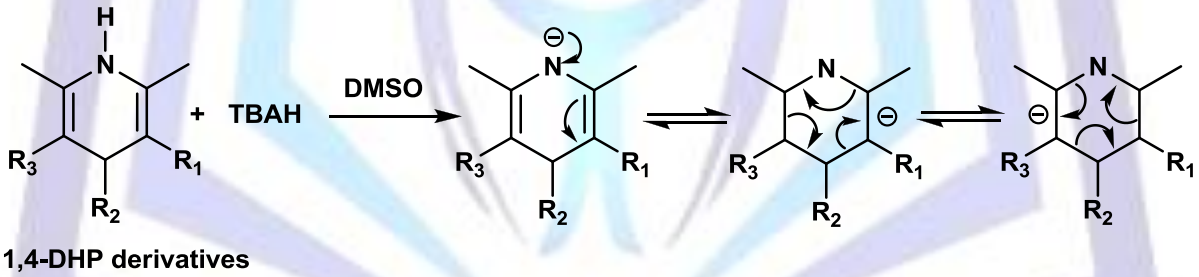


Fig 6. Job's plot for the reaction TBAH with NIF

Suggested reaction between the studied drugs and NBS:

Based on previous literatures, our experimental finding and the calculated molar ratios we suggested that the reaction mechanism is anion formation between acidic N-H and TBAH, as illustrated in **Scheme 1.** The aromatization of the 1,4-DHP ring is difficult under this conditions; that was established by the destructive effect of perchloric acid on the developed chromogen. The new finding in this study is the negative effect of nitro group on the reaction pass-way as it was reported in the previous explanations [6, 32,58].



Scheme 1. The suggested reaction pathway of reaction between 1,4-DHP and TBAH

VALIDATION OF PROPOSED METHODS

The developed procedures were fully validated according to USP XXVI [59] validation guidelines and International Conference on Harmonization (ICH) guidelines [60].

Linearity range, detection and quantification limits:

Calibration curves for the investigated drugs with TBAH method constructed by analyzing a series of different concentrations of the drug standard solutions. Linear relationships were found between the measured values of the absorbance intensities and the concentrations of the investigated drugs as indicated by the high correlation coefficients obtained (*r*), in the concentrations were ranged from 2.50-40.0 µg/mL. The limit of detection (LOD) and limit of quantitation (LOQ) were determined according to the IUPAC definitions [61] using the formula LOD or LOQ = $\kappa SD_{a/b}$; where κ = 3 for LOD and 10 for LOQ, SD_a is the standard deviation of the intercept and b is the slope. The regression equations for the results derived using the least square method and the obtained results summarized in **Table 1.**

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Table 1. Quantitative parameters and statistical data for the proposed method

Drug	Linear range µg/mL	*r*	Regression equation[1]	LOD Mg/mL	LOQ µg/mL	ε*×10^4 l/mol/m
NIF	2.5-35	0.996	y= 0.023x + 0.015	1.956	6.521	0.893
NIC	2.5-35	0.996	y= 0.021x + 0.010	0.750	2.500	1.137
NIM	5.0-40	0.999	y= 0.021x + 0.009	1.280	4.285	0.939
FEL	2.5-30	0.997	y= 0.031x + 0.014	1.354	4.516	1.370
AML	2.5-35	0.996	y= 0.024x + 0.010	1.125	3.751	1.460

[1]y: absorbance and x: Sample conc., µg/mL *ε: The molar absorptivity

Precision and accuracy:

The proposed method's precision was determined by carrying out replicate analysis of five separated solutions of the working standards at one concentration level. The relative standard deviations (RSD) of the results did not exceed 2%, for intra- and inter-day precision indicating a good repeatability and reproducibility with acceptable accuracy of the proposed method. **Table 2**

Table 2. Assay of five replicate samples of the studied drugs by TBAH at one concentration level

Drug	Conc., µg/mL	Accuracy % (n-5)	Precision RSD (%) (n=5)	
			Intra-day	Inter-day
NIF	20	91.1±0.004	0.38	1.6
NIC	25	96.4±0.003	0.73	1.8
NIM	15	100.0±0.004	0.63	1.2
FEL	20	95.1±0.003	0.53	1.4
AML	20	101.4±0.003	0.39	1.5

Robustness and Ruggedness

The proposed method's robustness was examined by evaluating the influence of small variation in some experimental parameters on its suitability and specificity. The changed parameters were the concentration of TBAH and the reaction time. It was found that none of these variables significantly affect the original data of the proposed method, **Table 3.**

Table 3. Robustness of the proposed method

Variation	Recovery % ± SD[a]				
	NIF[b]	NIC[b]	NIM[b]	FEL[b]	AML[b]
No variation	98.34±0.23	97.21±0.24	98.38±0.25	99.30±1.25	99.22±0.26
TBAH conc.					
24.75 mg/mL	99.40 ± 1.23	98.20 ± 1.12	97.27 ± 1.28	99.52±1.20	99.20±0.26
25.25 mg/mL	98.24 ± 1.22	98.11 ± 0.34	98.19 ± 0.47	99.23±1.14	99.51±1.18
Reaction time					
19 min	98.27±1.34	98.20±0.21	99.37±0.81	99.31±0.77	99.65±1.36
21 min	99.25±1.22	98.43±0.13	98.27±0.87	99.34±0.36	99.89±0.56

[a]Values are the mean of three determinations ± SD [b]Drugs conc., 20 µg/mL

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The proposed method's ruggedness was tested by applying the assay of the investigated drugs using the same conditions but using two different instruments at two different laboratories and different elapsed time, the results obtained were found to be reproducible as RSD did not exceed 2%, **Table 4.**

Table 4. Ruggedness of the proposed TBAH method

Drugs[b]	Recovery % ± SD[a]				
	Instrument		Inter-day variation		
	Shimadzu	Jenway	Day-1	Day-2	Day-3
NIF	99.24±0.89	99.21±1.24	98.32±0.23	97.22±1.56	99.62±0.51
NIC	99.66±1.17	99.99±1.18	97.27±0.24	99.08±1.35	98.81±0.44
NIM	99.92±1.27	99.10±0.66	98.30±0.13	99.24±1.33	98.40±0.69
FEL	98.17±1.22	99.11±0.18	99.13±1.25	98.51±0.56	98.49±1.56
AML	99.29±1.83	98.15±1.88	99.02±0.26	97.29±1.34	98.41±1.34

[a]Values are the mean of three determinations ± SD [b]Drugs conc., 20 µg/mL

Selectivity of the proposed method

The selectivity of the method was checked by monitoring a standard solutions of the drugs in the presence of other ingredients which present in the tablets and capsules, such as atenolol or metoprolol with NIF and FEL, respectively or excipients [62], the response was not different from that obtained in the calibration curves.

Application of the proposed method

Tablets and capsules were subjected to analysis for their contents from 1,4-DHP drugs using the proposed method, **Table 5.** The results were compared with those obtained from either official [63] or reported methods [14, 40], with respect to the accuracy (t-test) and precision (F-test). No significant differences were found between the calculated and theoretical values of t- and F-tests at 95% confidence level proving similar accuracy and precision in the analysis of the investigated drugs in their dosage forms.

Table 5. Determination of the studied drugs in their tablets and capsules using TBAH and official or reported methods

Product	Recovery % ± SD[a]		F-value[b]	t-value[b]
	Proposed method	Official or reported method[c]		
Epilate® capsules	97.43±0.12	99.52±0.11	2.98	1.67
Epilate Retard® tablets	99.45±0.17	98.51±0.13	2.13	1.20
Tenolat SR® capsules*	97.21±0.12	100.52±0.66	1.81	1.61
(Pelcard SR® capsules)[c]	98.56±0.02	99.74±0.11	1.62	2.24
Nimotop® tablets	99.48±0.15	98.61±0.19	1.49	1.93
Plendil® tablets	97.69±0.10	99.24±0.13	2.64	1.67
Plentopine® tablets	97.52±0.15	99.22±0.11	1.62	1.18
Logimax® tablets*	99.31±0.18	99.34±0.17	1.12	0.36
(Alkapress® tablets)[c]	98.40±0.19	99.21±0.12	4.69	1.80
(Myodura® tablets)[c]	98.24±0.13	98.31±0.11	1.39	0.16
(Amlodipine® tablets)[c]	99.23±0.11	97.20±0.12	1.00	1.23
(Regcor® tablets)[c]	99.39±0.12	99.24±0.16	1.14	0.20
(Vasonorm® tablets)[c]	98.33±0.16	98.41±0.19	1.14	0.17

[a]Mean of five determinations [b]Theoretical values for F and t at 95% confidence limit (n = 5) were 6.39 and 2.78, respectively. [c]Reported methods [14, 40] *Drugs that present in combination

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CONCLUSION

A yellow color that formed under the above mentioned conditions measured spectrophotometrically can be regarded as anion formation between acidic N-H in 1,4-DHP drugs and TBAH alkali. The new finding in this study is the negative effect of nitro group on the reaction pass-way as it reported in the previous explanations. The proposed method have the advantages of simplicity, sensitivity, selectivity and reproducibility, which satisfies the need for a rapid procedure for routine determination of 1,4-DHP drugs in bulk or in their dosage forms.

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