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SPECTROPHOTOMETRIC AND SPECTROFLUORIMETRIC DETERMINATION OF 1,4-DIHYDROPYRIDINE DRUGS USING POTASSIUM PERMANGANATE AND CERIUM (IV) AMMONIUM SULPHATE

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هذا البحث يختص بتعين أدوية ٤،١-ثنائى هيدروبيريدين بطرق بسيطة وحساسة الطريقة الاولى: بواسطة التحليل الطيفي عن طريق أكسدة هذة الادوية فى وسط حامضى باستخدام برمنجنات البوتاسيوم فى وجود حمض الكبريتيك وهى تعتمد على اختفاء لون برمنجنات البوتاسيوم والذى يقاس عند درجة امتصاص اللون عند قمة امتصاص عظمى طولها الموجى ٥٢٥ نانومتر نتيجة للتفاعل مع هذة الأدوية. والطريقة الثانية: بواسطة سلفات السريوم النشادرى رباعى التكافؤ فى وجود حمض الكبريتيك وقياس شدة اللصف نتيجة تكون السيريوم الثلاثى عند طول موجى ٣٥٥ نانومتر (الإثارة عند ٢٥٥ نانومتر). تم بدقة إختيار أنسب الظروف للتفاعل من حيث تركيزات المواد المتفاعلة والوسط المناسب للتفاعل والزمن المطلوب للتفاعل. وتم تطبيق الطريقة المقترحة بنجاح في تقدير الأدوية التى تم دراستها سواء في صورتها النقية أو في مستحضراتها الصيدلية المختلفة محققة درجة عالية من الدقة وذلك عند مقارنةً النتائج التى تم الحصول عليها بنتائج طرق دساتير الأدوية أو الدوريات العلمية.

Simple and sensitive spectrophotometric and spectrofluorimetric methods have been developed for determination of 1,4-dihydropyridine (1,4-DHP) drugs based on the oxidation of the investigated 1,4-DHP drugs with acidic $KMnO_4$ (method I) or Ce (IV) (method II). The first method is based on the decrease in the colour of the permanganate solution due to the presence of the studied drug was measured at 525 nm. And the second method is based on monitoring the fluorescence of the produced cerium (III) at emission 355 nm (excitation at 255 nm). All variables that affect the performance of the proposed methods were carefully studied and optimized. The analytical performance of the methods was validated according to International Conference of Harmonization guidelines. The proposed methods were applied successfully to the determination of the drugs in commercial tablets and capsules. The results of the proposed procedures were statistically and compared with those obtained by the reference methods.

INTRODUCTION

1,4-DHP drugs; namely nifedipine (NIF), nicardipine (NIC), nimodipine (NIM), felodipine (FEL) and amlodipine (AML) (Table 1); are primarily used for treatment of cardiovascular diseases such as hypertension, angina and some forms of cardiac arrhythmias. Recently, it has been suggested that these agents may be useful in other pathological states, such as seizures and central ischemic disorders[1&2] through their action on slow L-type channels and they have a greater selectivity for vascular smooth muscles than for myocardium muscles and therefore their main effect is vasodilatation. They are non-rate-limiting with little or no action at the SA or AV nodes and the negative inotropic activity

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

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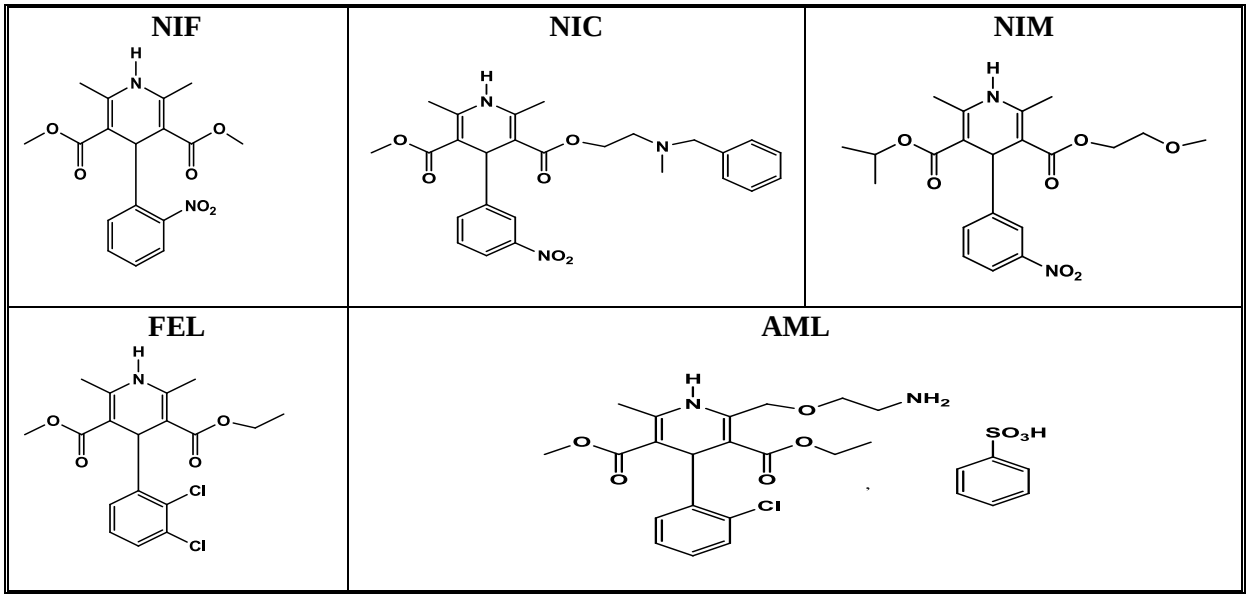


is rarely seen at therapeutic doses. NIM crosses the blood-brain barrier and is used in cerebral ischemia and some of the newer agents, such as AML and NIC, have the advantage that they show little interaction with other cardio-vascular drugs, such as digoxin or warfarin that are often used concomitantly with calcium channel antagonists[3].

Many analytical methods have been reported for detection and determination of 1,4-DHP drugs in bulk, in their pharmaceutical formulations, and/or in biological fluids. Several reviews[4&5] were published for analysis of NIF[4] NIM[5]. In the monographs of the British Pharmacopoeia[6], European Pharmacopoeia[7] and United States Pharmacopoeia[8], NIF, NIM, FEL and AML (pure and dosage forms) were assayed using redox titration or HPLC methods.

Other analytical techniques such as; titrimetric[9&10], spectrometric (Spectrophoto-metric[11-17] or spectrofluorimetric[18-23]), electro-chemical[24-26], high performance liquid chroma-tography[27-31] and gas chromatography[32-35] were reported.

EXPERIMENTAL

Instruments

- UV-1601 PC, UV-Visible Spectrophoto-meter (Shimadzu, Tokyo, Japan), with two matched 1 cm quartz sample cells.
- Jenway 6305, UV-Visible Spectrophoto-meter, U.K (Jenway LTD)
- Spectrofluorimeter RF 501 PC (Shimadzu, Tokyo, Japan), the slit width of both excitation and emission monochromators were set at 3 nm with 150 w xenon lamp.
- Analytical balance (Precisa, Presisa Instruments Ltd., Switzerland).
- Ultrasonic cleaner (Cole-Parmer, Chicago, U.S.A.).
- MLW type thermostatically controlled water bath (Memmert GmbH, Schwabach, Germany).
- Nanopure II water purification system (Barnstead / Thermolyne, Dubuque, IA, USA).

Chemicals and reagents

All chemical and reagents were of analytical grade and their solutions were prepared and diluted in double distilled water.

- **Solvents**; methanol, ethanol, acetone, acetonitrile, chloroform, ethylether and dimethylformamide (DMF), (El-Nasr Pharmaceutical Chemical Co., Abo-Zaabal, Egypt).

- **Additves**; glucose, lactose, sucrose, magnesium stearate, talc, starch and gum acacia, (El-Nasr Pharmaceutical Chemical Co., Abo-Zaabal, Egypt).
- **Reagents**
- Ferric chloride, potassium ferrocyanide, sodium hydroxide, potassim hydroxide, disodium hydrogen phosphate, sodium

bismuthate and acids (sulphuric, hydrochloric, nitric, perchloric, citric and acetic); all these chemicals were obtained from El-Nasr pharmaceuticals and chemicals Co., (Abo-Zaabal, Egypt).

- Potassium permanganate (El-Nasr Pharmaceutical Chemical Co., Abo-Zaabal, Egypt). 0.07% w/v was freshly prepared in distilled water. The solution was heated to boiling and kept on the steam bath for one hour and then filtered through a sintered glass filtering crucible. The solution was stored in a dark container.
- Cerium ammonium sulphate (IV) (Sigma Co. St. Louis, USA) 0.75 mg/ml was prepared by dissolving 75 mg Ce $(NH_4)_4(SO_4)_4.H_2O$ in 100 ml of 0.25 M of sulphuric acid. To avoid the presence of any Ce (III) with Ce (IV) solutions, 1 g sodium bismuthate was added to oxidize any Ce (III) if present. The excess sodium bismuthate was eliminated by filtration[36].
- Acid stock solutions for certain molarity of each of the following acids; acetic, hydrochloric, nitric, perchloric and sulphuric acids were freshly prepared in double distilled water.

Pure samples

Samples of cited drugs were generously supplied by their respective munufactures and the purities of authentic samples were checked by UV assay methods for investigated pure material[37]:

1- NIF and atenolol were obtained from Egyptian International Pharmaceutical Industries Co. [EIPICO], Cairo, Egypt).
2- NIC HCl was obtained from Global Napi (GNP, Cairo, Egypt).
3- NIM was obtained from Bayer Health Care, Cairo Egypt.
4- FEL was obtained from Astra Zenika, Cairo, Egypt.
5- AML besylate was obtained from T3A, Assuit, Egypt.
6- Metoprolol was purchased from Sigma (Sigma Chemical Co, St. Louis, USA).

Pharmaceutical formulations

1- Epilate® capsules and Epilate Retard® tablets (EIPICO) labeled to contain 10, 20 mg of NIF per tablet respectivly.
2- TenolatSR® capsules (Sigma/Tiba, Cairo, Egypt) labeled to contain 20, 50 mg of NIF and atenolol, per tablet respectivly.
3- PelcardSR® capsules (GNP /Wockhardt, Cairo, Egypt) labeled to contain 50 mg of NIC per tablet.
4- Nimotop® tablets (Bayer Health Care, Cairo, Egypt) labeled to contain 30 mg of NIM per tablet.
5- Plendil® tablets (Astra Zeneca, Cairo, Egypt) and Plentopine® tablets (Sinaph, Cairo, Egypt) labeled to contain 10 mg of FEL per tablet.
6- Logimax® tablets (Astra Zeneca, Cairo, Egypt) labeled to contain 5, 50 mg of FEL and metoprolol, per tablet respectivly.
7- Alkapress® tablets (Alkan pharm, Cairo, Egypt), Myodura® tablets (GNP / Wockhardt, Cairo, Egypt) and Vasonorm® tablets (Pharco, Alexandera, Egypt) labeled to contain 10 mg of AML per tablet.
8- Amlodipine® tablets (GNP / Wockhardt, Cairo, Egypt) and Regcor® tablets (EIPICO) labeled to contain 5 mg of AML per tablet.

Preparation of stock standard solution 1,4-DHP drugs

N.B. Stock standard 1,4-DHP solutions should be freshly prepared and kept in dark containers due to their photosensitivity[6].

For method I

An accurately weighed 50 mg of each of the studied drugs was transferred into a 100-ml calibrated flask, and dissolved in about 2 ml of conc. sulphuric acid. The contents of the flask were swirled and completed to volumes with 50% v/v of sulphuric acid. The working standard solutions were prepared by further dilution with distilled water to obtain concentrations covering the range of 2.50-30.0 µg/ml.

For method II

An accurately weighed amount of about 10 mg of each of the studied drugs was transferred into a 100-ml calibrated flask and dissolved in about 50 ml of ethanol. The contents of the flask were shaked well and completed to volume with the same solvent to provide a stock standard solution containing 100 µg/ml.

Preparation of pharmaceutical dosage forms

Tablets and capsules

An amount equivalent to 50 mg of the active ingredient of 20 finally powdered tablets or mixed 20 capsules content was weighed accurately, transferred into a beaker followed by 10 ml chloroform. The contents of the flask were swirled, sonicated for abut 5 min, then filtrated through a Whatmann No. 42 filter paper and washed with small amount of chloroform. The filtrate was evaporated to dryness and the residue was dissolved in 2 ml of sulphuric acid for method I or 10 ml ethanol for method II and completed quantitivly in a 100 ml volumetric flask by 50% sulphuric acid or ethanol for I and II respectevly.

Tablets and capsules containing binary drugs

20 tablets or the contents of 20 capsules were weighed, finely powdered. An accurately weighed quantity of the powdered tablets or capsules contents equivalent to 25 mg of the active ingredient was transferred into a 50-ml volumetric flask, dissolved in about 10 ml of chloroform (Tenolat SR® capsules), or in 10 ml diethylether (Logimax® tablets). The contents of the flask were swirled, sonicated for 5 min, then filtrated. The obtained filtrate was evaporated to dryness and the residue was dissolved in 2 ml of sulphuric acid for method I or 10 ml ethanol for method II and completed quantitivly in a 50 ml volumetric flask by 50% sulphuric acid or ethanol for method I and II respectevly.

General recommended procedures

An accurately measured one ml of the standard or sample solution was transferred into a 10-ml calibrated flask, then 1.0 ml of $KMnO_4$ (0.07% w/v in distilled water) method I or 1.0 ml of Ce (IV) method II was added. The solution was allowed to stand for 10 min or 15 min for I and II respectivly, then completed to the mark with bi-distilled water. The absorbance was measured at 525 nm and the relative fluorescence intensity (RFI) was measured at 355 nm (excitation at 255 nm) against reagent blanks treated similarly. For method I the positions of sample and blank cuvettes were exchanged; this was done in order to measure directly the decrease in absorption intensity (ΔA) that resulted from the presence of the drug in the sample.

Procedures for the determination of reaction stoichiometry

Job's method of continuous variation[38] was employed to established the stiochiometry of the reaction; Master equimolar solutions $3x10^{-3}$ M of both Ce (IV) and the investigated drugs 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-ml volumetric flasks, mixed well, and allowed to stand for 15 min. and completed as under general recomnded procedure.

RESULTS AND DISCUSSION

The main porpose of this study was to estabish simple spectrophotometric and spectrofluorimetric methods for the determination of the investigated 1,4-DHP.

In method I; the drugs were oxidazible by $KMnO_4$ in acidic solution this was evidented from the decrease in the violet colour at 525 nm of the $KMnO_4$ solution (Fig. 1). The decrease in colour (ΔA) was used as a measure for the concentration of the drugs in their solutions. In method II; the oxidation resulting in release of cerium (III) which measured at emission 355 nm (after excitation at 255 nm) (Fig. 2).

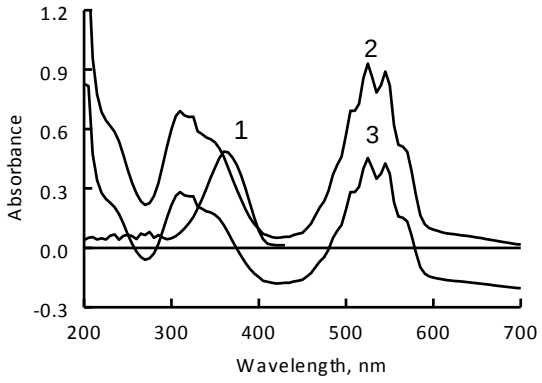


Fig. 1: Absorption spectra of (1) 20 µg/ml of FEL, (2) 0.07% w/v $KMnO_4$ and (3) their reaction product.

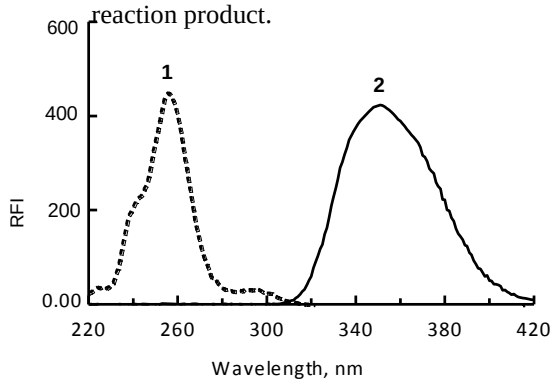


Fig. 2: Excitation (1) and emission (2) spectra of the reaction product of 0.5 µg/ml of NIC and 1.0 ml of 0.75 mg/ml Ce (IV) solution.

Optimization of the reaction variables

Effect of reagents concentration

The optimum absorbance intensity ≈ 0.9 was obtained when 1.0 ml of 0.07% w/v $KMnO_4$ solution was used for method I, further increase in the permanganate concentration had no effect on the reaction, but an increase in the blank absorbance.

Serial stock solutions of Ce (IV) in concentration range of 0.25-1.75 mg/ml were prepared. One ml of each concentration was added as in the general assay procedure. The obtained results, shown in figure 3, indicated that the higher fluorescence intensity was obtained when Ce (IV) concentration was 0.5-1.0 mg/ml. Above this concentration the intensity begins to decrease. Therefore 0.75 mg/ml Ce (IV) was selected for method II.

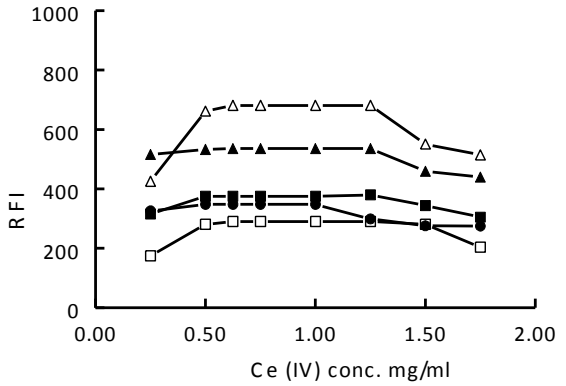


Fig. 3: Effect of Ce (IV) conc. on the RFI with 0.5 µg/ml of NIF (-□-), NIC (-■-), NIM (-▲-), FEL (-Δ-) and AML (-●-).

Effect of type and concentration of the acid

In solutions having hydrogen ion concentration of 0.5 M or greater, permanganate is reduced only to manganous ion; the increased acidity probably enhances the ease of protonation of such organic compounds and hence the rate of their oxidation[39]. Therfore oxidation reaction of the studied 1,4-DHP drugs by method I was performed in acid medium. And for method II the oxidation reaction was carried out in acid medium to avoid the precipitation of hydrated ceric oxide, $CeO_2.xH_2O$[40].

Table 2 shows that 50% Sulphuric acid gave the highest absorbance intenisty. Therefore, it was selected for further testing with $KMnO_4$ reagents and for method II, Also sulphuric acid gave the highest RFI, thus it was selected for the subsequent work (Table 3).

Figure 4 shows that the highest fluorescence intensity was obtained when sulphuric acid concentration was 0.20-0.3 M, above this concentration the intensity decreased. therfore 0.25 M sulphuric acid was used for the subsequent work.

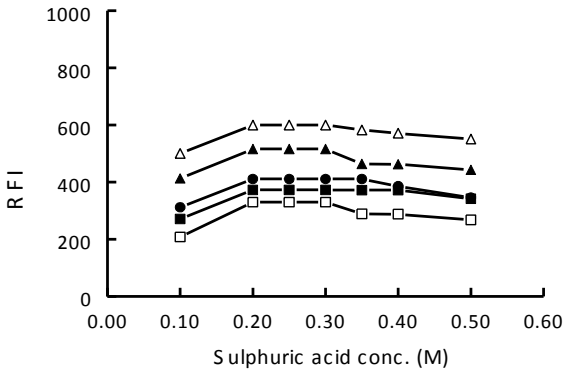


Fig. 4: Effect of Sulphuric acid conc. on RFI between Ce (IV) and 0.5 µg/ml of each of NIF (-□-), NIC (-■-), NIM (-▲-), FEL (-Δ-) and AML (-●-).

Effect of reaction time and temperature

The redox process at the first few seconds is very slow; nevertheless, succeeding portion of permanganate reacts more and more rapidly until the reaction becomes essentially instantaneous. This behaviour is typical for an autocatalytic process, in which one of the reaction products function as a catalyst for the next steps catalyst for the next steps[39]. The results revealed that the reaction was

completed within 5-10 min, but for more precision measurements were carried out after 10 and 15 min for method I and II respectively (Figs. 5 and 6).

Table 2: Effect of acids type on absorption intensity of the reaction of 1.0 ml of 0.07% w/v $KMnO_4$ with 20 µg/ml of each drugs.

Acid type[a]	Absorbance difference				
	NIF	NIC	NIM	FEL	AML
Acetic	0.165	0.217	0.195	0.211	0.211
Hydrochloric	0.223	0.224	0.215	0.210	0.322
Nitric	0.332	0.311	0.312	0.310	0.310
Perchloric	0.411	0.311	0.325	0.345	0.382
Sulphuric	0.565	0.511	0.425	0.515	0.460

[a] Acid concentration used 5.7 M.

Table 3: Effect of acid type on the RFI of 1,4-DHP drugs (0.5 µg/ml) with Ce (IV) solution.

Acid type[a]	R FI				
	NIF	NIC	NIM	FEL	AML
Acetic acid	234	241	370	462	233
Sulphuric acid	315	380	527	620	331

[a] Acid concentration used 0.25 M.

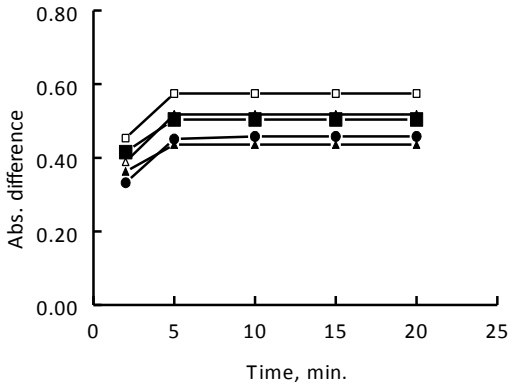


Fig. 5: Effect of time on the reaction of $KMnO_4$ (0.07% w/v) with 20 µg/ml NIF (-□-), NIC (-■-), NIM (-▲-), FEL(-Δ-) and AML (-●-).

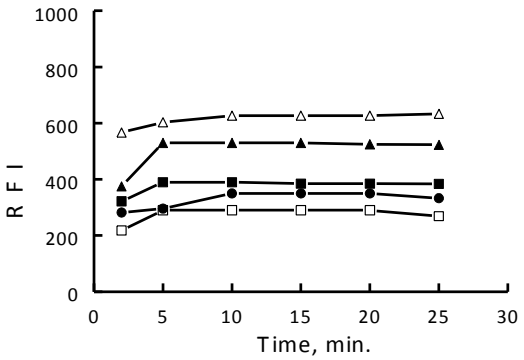


Fig. 6: Effect of the reaction time on RFI between Ce (IV) and 0.5 µg/ml of each of NIF (-□-), NIC (-■--), NIM (-▲--), FEL (-Δ-) and AML (-●-).

Since the reaction was carried out in in high sulphuric acid concentration, the heat generated was found to be sufficient for the reaction to proceed quickly with no more heating, for method I and for method II elevated temperature ranging from 40-60°C in a thermostatically controlled water bath for different times had no significant accelerating effect on the reaction time and subsequently RFI.

Stability of the chromogen or fluorophore formed

The effect of time on the stability of the drug-permanganate or Ce (IV) reaction product was studied for all drugs by monitoring the difference in the absorption intensities (ΔA) or

(RFI) at different time intervals after diluting the reaction mixture. The results show that ΔA values or RFI were stable for at least 30 min. This gives the advantage of comfortable measuring at any time within that period without any changes in the values.

Effect of diluting solvents

Dilution of the reaction mixture in method I with different solvents showed that both the position of λ_{max} and the difference of absorption intensities were influenced. The highest readings were obtained when bi-distilled water was used as a diluting solvent (Tables 4 and 5). The absorbance values were not correlated well with the dielectric constants of the solvents used for dilution.

Distilled water was recommended as safe and cheap solvent for further readings for method I and II.

Stoichiomerty of the reaction

Molar ratio of the reaction between Ce (IV) and 1,4-DHP drugs (as example) was studied using Job's method of continuous variation, $3x10^{-3}$ M of both Ce (IV) and 1,4-DHP drugs were prepared. The study revealed that the ratio between the investigated drugs : Ce (IV) was 1:4 (Fig. 7).

Reaction mechanism

The oxidation of 1,4-DHP drugs were suggested to occur through aromatization of the 1,4-DHP ring[42] (Scheme 1).

Validation of the proposed methods

The developed procedures were fully validated according to USP *XXV*[43] validation guidelines and International Conference on Harmonization (ICH)[44] guidelines.

Linearity range, detection and quantitation limits

Under the above mentioned optimum conditions, the calibration graphs for the investigated drugs; correlating the decrease in the absorption intensities (ΔA) or increase in (RFI) with the corresponding concentrations of the drugs were constructed. Regression analysis for the results was performed using least-square method (Figs. 8 and 9).

With respect to all drugs, calibration plots were found to be linear as indicated by the high correlation coefficients obtained 0.9955-0.9999 and 0.9982-0.9999 and the small intercepts in

Table 4: Effect of different solvents on the difference in absorption intensity of the reaction products of the drugs (20 µg/ml) with $KMNO_4$

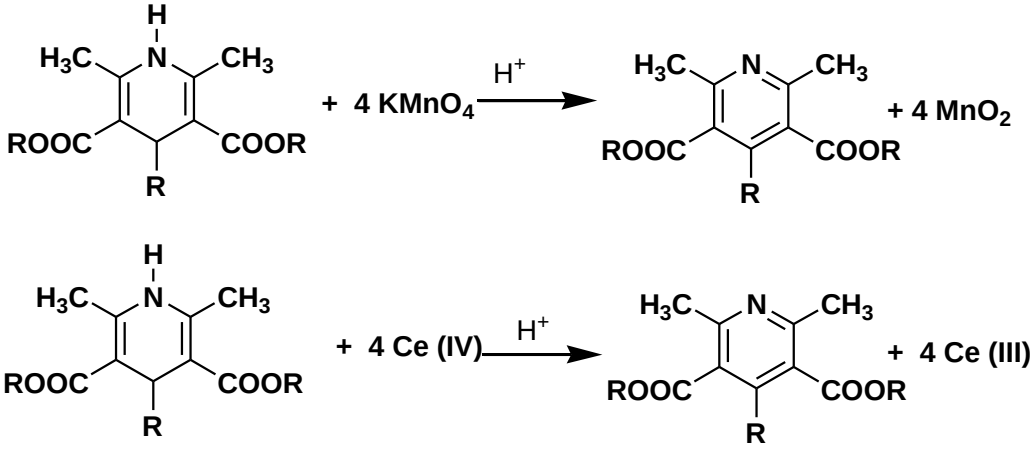
Solvent	λ_{max}	DEC[a]	Abs. difference				
			NIF	NIC	NIM	FEL	AML
Acetone	530	73.50	0.322	0.250	0.305	0.414	0.346
Ethanol	522	24.30	0.120	0.254	0.325	0.430	0.351
Methanol	525	32.63	0.250	0.260	0.335	0.453	0.391
Propan-1-ol	527	20.70	0.312	0.240	0.314	0.425	0.387
Water	525	78.54	0.566	0.495	0.430	0.525	0.454

Table 5: Effect of diluting solvents on RFI of 1, 4-DHP drugs with Ce (IV).

Solvent	DEC[a]	RFI				
		NIF 0.5 µg/ml	NIC 0.5 µg/ml	NIM 0.25 µg/ml	FEL 0.25 µg/ml	AML 0.5 µg/ml
Acetone	73.50	18	26	15	23	25
Ethanol	24.30	46	62	32	47	46
Methanol	32.63	32	44	22	35	36

Propan-1-ol	20.70	62	87	47	63	65
Water	78.54	318	375	537	621	152

[a]DEC: dielectric constant[41].



Scheme 1: Suggested mechanism for oxidation of 1, 4-DHP drugs by $KMnO_4$ and Ce (IV) in acidic medium.

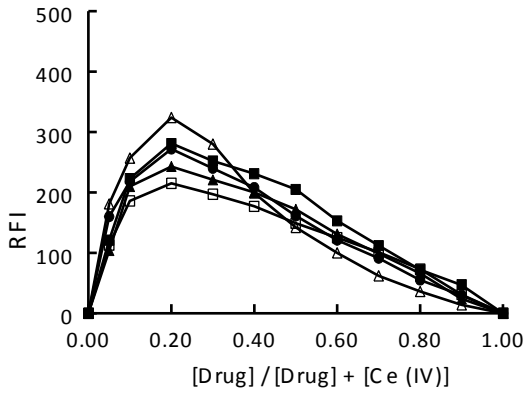


Fig. 7: Job's plot for the reaction between Ce (IV) and NIF (-□-), NIC (-■-), NIM (-▲-), FEL(-Δ-) and AML (-●-) of the same molar concentration.

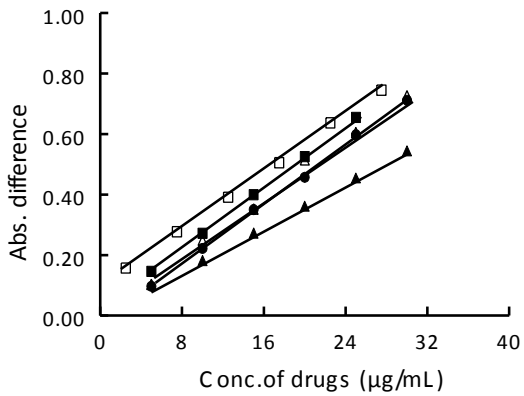


Fig. 8: Calibration curves obtained from the reactions of NIF (-□-), NIC (-■--), NIM (-▲--), FEL (-Δ-) and AML (-●-) with $KMnO_4$ (0.070% w/v).

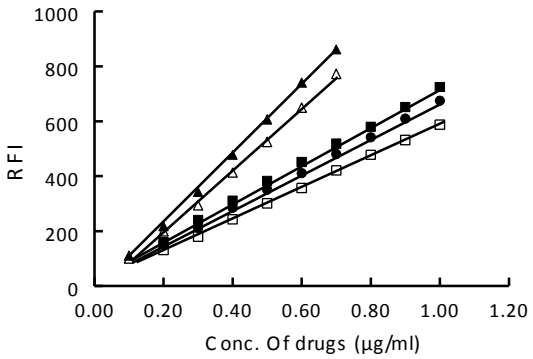


Fig. 9: Linear relation between RFI and NIF (-□-), NIC (-■-), NIM(-▲-), FEL(-Δ-) and AML (-●-) with the fluorimetric method.

the general concentration range of 2.50-30.0 and 0.10-1.00 µg/ml for method I and II respectively (Tables 6 and 7). The limits of detection (LOD) and limits of quantitation (LOQ) were determined according to the IUPAC definitions[45] using the formula: LOD or LOQ = κSDa/b; where κ = 3.3 for LOD and 10 for LOQ, SD_a is the standard deviation of the intercept, and b is the slope.

Precision

The precision of the proposed methods was determined by carrying out replicate analysis of five separate solutions of the working standards at one concentration level of each drug according to USP XXV validation guidelines[43].

The relative standard deviations of the result did not exceed 2%, indicating the good repeatability of the proposed methods (Tables 8 and 9). This level of precision is adequate for routine analysis of the investigated drugs.

Interference studies

The suggested methods were not selective as they depend on the use of non differenciating strong oxidizing agents. So the presence of reducing substance in the same dosage, combined drugs or excipients, may interfere with the results of this method. But fortunately; the interference that can arise from the presence of the second drug (atenolol or metoprolol), co-administrated drugs (warfarin or digoxin) and common excipients such as; starch, gum acacia, magnesium stearate and talc could be eliminated by physical separation through filtration or selective solvent extraction. While the interference from combined drugs or excipients and co-administrated drugs in fluorimetric method This may be attributed to the great sensitivity of the method that necessitated the dilution of the sample and all these additives are almost insoluble in the organic solvent used and consequently that dilution for sample solution make the exipients beyond their interference capabilities.

Robustness and Ruggedness

It was found that non of variables significantly affect the methods (Tables 10 and 11). The recovery values provided an indication for the reliability of the proposed methods.

Ruggedness was tested by applying the two proposed methods to the assay of the same samples of the investigated 1,4-DHP using the same operational conditions but at different elapsed times and using two different instruments. Results obtained from day-to-day variations were found to be reproducible, as RSD did not exceed 2% (Tables 12 and 13).

Analysis of pharmaceutical dosage forms

The proposed methods have been successfully aplied to the determination of the studied drugs in their pharmaceutical formulations tablets, and capsules and compared by the official[6] or reported methods[46&47] (Tables 14 and 15).

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.

Table 6: Quantitative parameters for the analysis of 1,4-DHP by the proposed method I.

Drug	Linear range(μg/ml)	Intercept ± SD	Slope ± SD	Corr. coeff. (r)	$\varepsilon\times10^{-4}$ ($lmol^{-1}cm^{-1}$)	LOD (μg/ml)	LOQ (μg/ml)
NIF	2.50-27.5	0.098±0.005	0.024±0.0003	0.9995	0.9993	0.60	2.0
NIC	5.00-25.0	0.018±0.001	0.025±0.0001	0.9999	1.3545	0.12	0.41
NIM	5.00-30.0	0.004±0.008	0.018±0.0002	0.9994	0.7448	1.33	4.40
FEL	5.00-30.0	-0.010±0.016	0.025±0.0008	0.9955	0.9876	1.92	6.40
AML	5.00-30.0	-0.024±0.006	0.025±0.0003	0.9993	1.2958	0.72	2.40

Table 7: Quantitative parameters for the analysis of 1, 4-DHP by the proposed method II.

Drug	Linear range(μg/ml)	Intercept ± SD	Slope ± SD	Corr. coeff. (r)	LOD (μg/ml)	LOQ (μg/ml)
NIF	0.20-1.00	11.61±2.480	579.0±4.348	0.9996	0.0147	0.0490

NIC	0.20-1.00	-28.29±11.00	1126±24.59	0.9976	0.0293	0.0976
NIM	0.10-0.70	30.30±4.573	694.5±7.00	0.9993	0.0197	0.0658
FEL	0.10-0.70	-29.00±7.174	1273±16.04	0.9992	0.0169	0.0563
AML	0.20-1.00	19.20±2.348	656.3±3.649	0.9998	0.0108	0.0363

Table 8: Assay of five replicate samples of the studied drugs by method I.

Drug	Conc. (µg/ml)	Abs. difference					Mean ± SD	RSD
		1	2	3	4	5		
NIF	20.0	0.568	0.573	0.558	0.549	0.575	0.565 ± 0.009	1.73
NIC	20.0	0.531	0.516	0.511	0.513	0.523	0.519 ± 0.007	1.41
NIM	20.0	0.461	0.464	0.463	0.459	0.459	0.461 ± 0.002	0.44
FEL	20.0	0.515	0.521	0.523	0.518	0.516	0.517 ± 0.003	0.58
AML	20.0	0.453	0.459	0.455	0.458	0.451	0.455 ± 0.003	0.66

Table 9: Assay of five replicate samples of the studied drugs by method II.

Drug	Conc. (µg/ml)	RFI					Mean ± SD	RSD
		1	2	3	4	5		
NIF	0.6	341	343	345	342	340	342 ± 1.7	0.5
NIC	0.7	419	416	418	417	420	418 ± 1.4	0.3
NIM	0.5	510	509	512	514	513	511 ± 1.9	0.4
FEL	0.5	621	622	623	627	625	623 ± 2.2	0.3
AML	0.6	402	403	400	405	408	403 ± 2.7	0.7

Table 10: Robustness of the proposed method I for analysis of (20 µg/ml) of investigated drugs.

Variation	Recovery % ± SD [a]				
	NIF	NIC	NIM	FEL	AML
No variation	98.32 ± 1.23	98.8 ± 0.26	99.32 ± 1.75	99.24 ± 1.25	99.41 ± 1.27
$KMnO_4$ conc.					
0.069% w/v	98.34 ± 0.43	99.21 ± 0.33	97.92 ± 1.16	97.86 ± 0.89	99.23 ± 1.34
0.071% w/v	98.36 ± 1.43	99.40 ± 0.45	98.71 ± 0.56	98.61 ± 1.34	99.30 ± 1.24
Reaction time					
9 min.	98.56 ± 1.22	98.72 ± 0.02	99.25 ± 1.56	98.27 ± 1.23	98.86 ± 1.35
11 min.	99.42 ± 1.33	99.24 ± 1.36	99.81 ± 0.46	99.40 ± 1.22	99.41 ± 1.33

[a] Values are the mean of three determinations ± SD.

Table 11: Influence of small variations in the assay conditions using fluorimetric method on the suitability test parameters and sensitivity.

Variation	Recovery % ± SD[a]				
	NIF 10 µg/ml	NIC 10 µg/ml	NIM 5 µg/ml	FEL 5 µg/ml	AML 10 µg/ml
No variation	99.60 ± 0.61	99.70 ± 0.71	99.10 ± 0.60	98.30 ± 0.82	99.60 ± 0.93
Ce (IV) conc.					
0.74 mg/ml	98.74 ± 0.95	98.55 ± 1.05	98.79 ± 0.94	97.10 ± 0.91	98.30 ± 1.12
0.76 mg/ml	97.15 ± 0.77	98.48 ± 0.88	99.35 ± 0.89	98.20 ± 0.71	98.24 ± 1.10
Sulphuric cid conc.					
0.24 M	99.75 ± 0.62	98.79 ± 0.82	98.15 ± 0.75	97.10 ± 0.78	99.30 ± 1.12
0.26 M	99.30 ± 0.91	98.86 ± 0.90	98.75 ± 0.82	99.20 ± 0.91	97.30 ± 1.10
Reaction time					
14 min	99.25 ± 0.17	99.25 ± 0.94	98.15 ± 0.81	98.20 ± 0.98	97.30 ± 1.16
16 min	98.90 ± 0.87	99.75 ± 0.77	98.75 ± 0.77	99.30 ± 0.88	98.60 ± 1.28

[a] Values are the mean of three determinations ± SD.

Table 12: Ruggedness of the proposed method I for analysis of 1, 4-DHP by acidic $KMnO_4$.

Drug	Recovery % ± SD [a]				
	Instrument		Inter-day variation		
	Shimadzu	Jenway	Day-1	Day-2	Day-3
NIF	98.32 ± 0.33	98.14 ± 1.25	98.13 ± 0.42	99.31 ± 0.66	99.63 ± 1.13
NIC	99.21 ± 1.33	99.46 ± 1.25	97.45 ± 1.52	99.54 ± 1.13	98.42 ± 0.27
NIM	99.31 ± 1.48	98.33 ± 1.34	99.31 ± 1.34	99.40 ± 1.45	99.26 ± 1.44
FEL	98.43 ± 0.89	99.11 ± 1.22	98.36 ± 1.22	99.21 ± 1.46	99.16 ± 0.25
AML	99.30 ± 1.16	98.59 ± 1.25	99.38 ± 1.02	99.15 ± 0.02	98.14 ± 0.16

Table 13: Ruggedness of the proposed spectrofluorimetric method.

Drug	Recovery % ± SD[a]				
	Intra-day variation		Inter-day variation		
	At morning	At evening	Day-1	Day-2	Day-3
NIF	98.26 ± 1.11	99.12 ± 0.35	98.79 ± 0.94	97.10 ± 0.91	99.30 ± 1.12
NIC	99.11 ± 1.26	98.36 ± 1.55	97.79 ± 0.94	98.43 ± 1.33	98.77 ± 0.89
NIM	99.29 ± 0.41	99.13 ± 1.40	98.75 ± 0.82	99.10 ± 0.91	99.63 ± 1.10
FEL	99.15 ± 0.33	99.47 ± 1.21	98.25 ± 0.79	99.33 ± 0.88	97.30 ± 1.16
AML	98.13 ± 1.16	99.10 ± 0.67	99.10 ± 0.60	100.30 ± 0.82	99.60 ± 0.93

[a]Values are the mean of three determinations ± SD.

Table 14: Determination of the studied drugs in their pharmaceutical formulations using method I and official or reported methods.

Product	Recovery % ± SD[a]		F-value[b]	t-value[b]
	Proposed method	Official or reported method[c]		
Epilate® capsules	99.41 ± 0.19	99.52 ± 0.11	2.98	0.35
Epilate Retard® tablets	98.42 ± 0.20	98.51 ± 0.13	2.13	0.18
Tenolat SR® capsules*	98.50 ± 0.16	100.52 ± 0.66	1.22	1.84
(Pelcard SR® capsules)[c]	99.41 ± 0.23	99.74 ± 0.11	1.62	0.05
Nimotop® tablets	99.35 ± 0.16	98.61 ± 0.19	1.49	1.28
Plendil® tablets	99.24 ± 0.41	99.24 ± 0.13	2.64	0.51
Plentopine® tablets	99.42 ± 0.17	99.22 ± 0.11	1.62	0.03
Logimax® tablets*	99.31 ± 0.18	99.34 ± 0.17	1.12	0.37
(Alkapress® tablets) [c]	98.43 ± 0.16	99.21 ± 0.12	2.69	1.39
(Myodura® tablets) [c]	98.21 ± 0.13	98.31 ± 0.11	1.39	0.17
(Amlodipine® tablets) [c]	99.32 ± 0.11	97.20 ± 0.12	1.00	1.12
(Regcor® tablets) [c]	99.20 ± 0.15	99.24 ± 0.16	1.14	0.13
(Vasonorm® tablets) [c]	98.34 ± 0.18	98.41 ± 0.19	1.14	0.17

[a]Values are the mean of five determinations .
[b]Theoretical values for F and t at 95% confidence limit (n= 5) were were 6.39 and 2.78, respectively.
[c]Reported methods[46&47].

Table 15: Determination of the studied drugs in their pharmaceutical formulations using the method II and official methods.

Product	Recovery % ± SD[a]		F-value[b]	t-value[b]
	Proposed method	Official or reported method[c]		
Epilate® capsules	99.27 ± 0.40	99.52 ± 0.11	2.51	1.41
EpilateRetard® tablets	99.20 ± 0.20	98.51 ± 0.13	1.59	1.60
TenolatSR® capsules*	99.19 ± 0.84	100.52 ± 0.66	4.29	1.47
(PelcardSR®capsules)[c]	99.15 ± 0.54	99.74 ± 0.11	1.76	1.84
Nimotop® tablets	99.11 ± 0.50	98.61 ± 0.19	1.01	1.14
Plendil® tablets	99.92 ± 0.48	99.24 ± 0.13	1.28	1.18
Plentopine® tablets	99.48 ± 0.42	99.22 ± 0.11	1.76	1.35
Logimax® tablets*	97.76 ± 0.34	99.34 ± 0.17	1.38	1.45
(Alkapress® tablets) [c]	99.39 ± 0.30	99.21 ± 0.12	1.87	1.74
(Myodura®tablets) [c]	98.96 ± 0.63	98.31 ± 0.11	1.53	1.64
(Amlodipine® tablets) [c]	99.33 ± 0.61	97.20 ± 0.12	2.52	1.44
(Regcor® tablets) [c]	98.58 ± 0.55	99.24 ± 0.16	3.20	1.06
(Vasonorm® tablets) [c]	98.76 ± 0.64	98.41 ± 0.19	2.57	1.84

[a]Values are the mean of five determinations .
[b]Theoretical values for F and t at 95% confidence limit (n= 5) were were 6.39 and 2.78, respectively.

[c]Reported methods[46&47].

Conclusion

In this work a simple, sensitive, precise, robust and accurate spectroscopic methods were described. Fortunately, all the analytical reagents used are inexpensive, have excellent shelf life, and are available in any analytical laboratory. In addition, the interference from other drugs can be eliminated by a simple one-step extraction method.

The proposed methods are of great values in quality control determinations of the cited drugs because of its adequate accuracy, reliability, low cost, and also because the instruments used were inexpensive and non-sophisticated, critical reagents are not required.

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