SPECTROPHOTOMETRIC AND SPECTROFLUORIMETRIC DETERMINATION OF CINNARIZINE AND FLUNARIZINE DIHYDROCHLORIDE IN PURE AND DOSAGE FROMS.

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Abstract:

Simple, rapid and reproducible spectrophotometric and spectrofluorimetric methods for quantitative determination of cinnarizine (CINN) and flunarizine dihydrochloride (FLUN) in pure and pharmaceutical formulations have been developed. The spectrophotometric methods were performed using two analytical reagents, picric acid and 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ). Reaction of picric acid with CINN and FLUN in chloroform yields yellow picrates with analytically useful maximum at 402.8 nm. Obedience of the formed chromogens to Beer`s law were found over the concentration ranges 12-60 µg ml^{-1} , 12-65 µg ml^{-1} with mean percentage recoveries 99.96 ± 0.70 and 100.16 ± 0.94 for CINN and FLUN, respectively. In addition, the charge transfer complex reaction between FLUN as n-donor and 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) as π- acceptor was applied for the estimation of FLUN. The formed highly colored radical ion exhibits maximum absorbance at 460 nm. Beer`s law was obeyed over the concentration range 30-175 µg ml^{-1} with a mean percentage recovery 100.13 ± 1.00 for FLUN. The spectrofluorimetric method was carried out by measuring the native fluorescence intensity of CINN and FLUN in methanol / 0.05N sulphuric acid medium (1:1) at 305 nm and 298 nm with excitation at 264 nm and 254 nm for CINN and FLUN, respectively. Linear correlations were obtained within the concentration ranges 0.2- 4 µg ml^{-1} and 0.2- 5 µg ml^{-1} with mean percentage recoveries 100.18 ± 0.71 and 99.76 ± 0.74 for CINN and FLUN, respectively. The proposed methods were successfully applied to the analysis of pharmaceutical formulations containing the above drugs with no interference from other drugs or dosage form additives. The validity of these methods was tested by the recovery studies of standard addition, which were found to be satisfactory. The percentage recoveries obtained have been in accordance with those given by the reference methods.

Keywords:

Spectrophotometry,spectrofluorimetry, cinnarizine, flunarizine, picric acid, DDQ.

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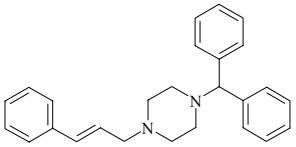
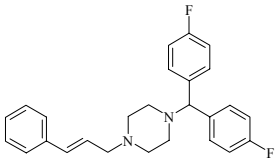
INTRODUCTION

Cinnarizine (CINN), [I] and its difluorinated analog flunarizine dihydrochloride (FLUN), [2] are known histamine H_1- receptor antagonists. They have selective calcium entry blocking activity and are widely used in cerebral and peripheral vascular disorders[1]. CINN is officially in the B.P.[2] and Eur.P.[3], whereas FLUN is officially available in Eur.P.[3].



[I] Cinnarizine



[II] Flunarizine

Several analytical techniques for the determination of CINN have been appeared in literature including titrimetry [2,3], spectrophotometry [4-9], derivative spectrophotometry [10-13] and derivative ratio spectrophotometry [14]. Chromatographic methods were also reported, such as HPLC [10,15-18], TLC [10,19] and GC [20]. An electrochemical method was described [21]. Chemometric techniques were also applied [13,14]. Several chromatographic methods for the analysis of FLUN in biological fluids including HPLC [22-27] and GC [20,28] were presented. Estimation of FLUN in pharmaceutically dosage forms by titrimetry [3], spectrophotometry [29-33], derivative spectrophotometry [34], HPLC [35] and electrochemical methods[34,36,37] was also reported.

Depending on the fact, that spectroscopic methods of analysis are usually rapid, sensitive and have the advantage of being inexpensive, the present work is directed to introduce simple spectroscopic methods for the determination of CINN and FLUN in their pure and pharmaceutical formulations.

EXPERIMENTAL

I. Instrumentation

1-SHIMADZU uv-visible recording spectrophotometer (Japan). Model UV 265 with 1-cm quartz cuvettes.

2- BIO-TEK Spectrofluorometer (Italy). Model SFM 25 with 1-cm quartz cuvettes connected to IBM compatible computer fitted with WIND 25 spectroscopy software for Windows (LabControl GmbH. BIO-TEK INSTRUMENTS).

II. Reagents and Chemicals

All chemicals used were of analytical grade and the solvents were of spectroscopic grade.

- Chloroform (Prolabo).
- Picric acid (E. Merck , Germany). 0.025% w/v solution in chloroform.
- Methanol (E. Merck , Germany).
- DDQ (E. Merck , Germany). 0.1% w/v freshly prepared solution in methanol.
- Sulphuric acid (Prolabo). 0.05N solution in distilled water.
- Sodium hydroxide (El-Nasr / Prolabo). 1N solution in distilled water.
- Anhydrous sodium sulphate (El-Nasr / Prolabo).

III. Samples

A) Pure Samples:

1) CINN and Piracetam: they were kindly supplied by Misr Company for Pharmaceutical Industries, Cairo, Egypt. Purity of these standards was 99.60 and 99.10 % for CINN and Piracetam, respectively as certified by the pharm. company.
2) FLUN: It was kindly supplied by MINAPHARM, Cairo, Egypt. Its purity was 99.85% as stated by the pharm. company.

B) Market Samples:

1) Stugeron tablets ^{R}: batch number 021061 packed by MINAPHARM, Cairo, Egypt under licence of JANSSEN PHARMACEUTICA, Belgium. It was labeled to contain 25 mg of CINN per tablet.
2) Cinaretam capsules^{R}: batch number 805072 from Misr Company for Pharmaceutical Industries, Cairo, Egypt. It is labeled to contain 25 and 400 mg per capsule for CINN and Piracetam, respectively.
3) Seblium capsules ^{R}: batch number 020822 packed by MINAPHARM, Cairo, Egypt under licence of JANSSEN PHARMACEUTICA, Belgium. It was labeled to contain 5 mg of FLUN base per capsule.

IV. Standard Preparations

- For the spectrophotometric methods

1) Stock Standard Solutions:

a. Standard stock solution of CINN 1 mg ml^{-1} was prepared by dissolving 25 mg of standard CINN in 25 ml chloroform.

b. Standard stock solution of Piracetam 16 mg ml^{-1} was prepared by dissolving 400 mg of standard Piracetam in 25 ml chloroform.

c. Standard stock solution of FLUN 1 mg ml^{-1} was prepared by dissolving an accurately weighed amount of the drug salt equivalent to 50 mg of drug base with 50 ml water in a separatory funnel. The solution was alkalinized with 10 ml 1 N sodium hydroxide solution and extracted with 5 successive 10 ml portions of chloroform. The chloroformic extracts were dried over anhydrous sodium sulphate, collected in a 50 ml volumetric flask and completed to volume with chloroform.

2) Working Standard Solutions:

- For Picric acid method

An aliquot of each standard stock solution (10ml) was diluted to 100 ml with chloroform to obtain a concentration of 100 µg ml^{-1} CINN, 1600 µg ml^{-1} Piracetam and 100 µg ml^{-1} FLUN. This solution was used as the working standard for the picric acid method.

For DDQ method

An aliquot of FLUN standard stock solution 1 mg ml^{-1} (25ml) was evaporated to dryness on a water bath. The residue was dissolved and diluted in a 100 ml volumetric flask with methanol to give a concentration of 250 µg ml^{-1} FLUN. This solution was used as the working standard for the DDQ method.

- For the spectrofluorimetric method

1) Stock Standard Solutions:

Standard stock solutions of CINN 0.1 mg ml^{-1}, Piracetam 1.6 mg ml^{-1} and FLUN 0.1 mg ml^{-1} were prepared by dissolving 10 mg standard CINN, 160 mg standard Piracetam and 10 mg standard FLUN in 100 ml of methanol / 0.05 N H_2SO_4 mixture (1:1).

2) Working Standard Solutions:

An aliquot of each standard stock solution (10ml) was diluted to 100 ml with the same solvent to obtain a concentration of 10 µg ml^{-1} CINN, 160 µg ml^{-1} Piracetam and 10 µg ml^{-1} FLUN. This solution was used as the working standard for the spectrofluorimetric method.

.. Laboratory Prepared mixtures of CINN and Piracetam:

- For Picric acid spectrophotometric method:

Accurately measured aliquots of CINN and Piracetam working standard solutions (100µg ml^{-1}), and (1600µg ml^{-1}) prepared for the picric acid method equivalent to 120-600 µg and 1920-9600 µg of CINN and Piracetam, respectively were transferred and mixed into a series of 10 ml volumetric flasks. These solutions were used as the laboratory prepared mixtures for the picric acid spectrophotometric method.

- For the Spectrofluorimetric method:

Accurately measured aliquots of CINN and Piracetam working standard solutions (10µg ml^{-1}), and (160µg ml^{-1}) prepared for the spectrofluorimetric method equivalent to 2- 40 µg and 32-640 µg of CINN and Piracetam, respectively were transferred and mixed into a series of 10 ml volumetric flasks. These solutions were used as the laboratory prepared mixtures for the spectrofluorimetric method.

V. Procedures

- Linearity

Spectrophotometric methods:

Picric acid method

Accurately measured aliquots of CINN and FLUN working standard solutions (100µg ml^{-1}) prepared for the picric acid method equivalent to 120-600 µg and 120-650 µg of CINN and FLUN, respectively were transferred into two series of 10 ml volumetric flasks. Each flask was treated with 2 ml picric acid reagent (0.025%w/v) and the volume was completed to the mark with chloroform. The absorbance of the yellow colored solution obtained was recorded at λ = 402.8 nm against blank solution prepared similarly. Calibration curves were obtained by plotting absorbance against drug concentrations and regression equations were computed.

DDQ method

Accurately measured aliquots of FLUN working standard solution (250µg ml^{-1}) prepared for the DDQ method equivalent to 300-1750 µg of FLUN, were transferred into a series of 10 ml volumetric flasks. Each flask was treated with 2 ml DDQ reagent (0.1%w/v) and allowed to stand for 10 minutes at room temperature. The volume was then adjusted to the mark with methanol and the absorbance of the colored solution obtained was measured at λ = 460 nm against blank solution prepared similarly. A calibration curve was constructed by plotting absorbance versus drug concentrations and regression equation was computed.

Spectrofluorimetric method:

Accurately measured aliquots of CINN and FLUN working standard solution (10µg ml^{-1}) prepared for the spectrofluorimetric method equivalent to 2-40 µg and 2-50 µg of CINN and FLUN, respectively were transferred into two series of 10 ml volumetric flasks and the volume was completed to the mark with methanol / 0.05 N H_2SO_4 mixture (1:1). The relative fluorescence intensities were recorded at λ_{em} = 305 nm, 298 nm applying λ_{ex}= 264 nm, 254 nm against blank [methanol / 0.05 N H_2SO_4 mixture (1:1)] for CINN and FLUN, respectively. A calibration graph for each drug was constructed by correlating the corresponding relative fluorescence intensities versus the corresponding drug concentrations and regression equations were computed.

- Analysis of CINN in laboratory prepared mixtures with Piracetam:

Spectrophotometric *(picric acid)* method*:*

To each flask of the laboratory prepared mixtures, 2 ml of picric acid reagent (0.025%w/v) was added and the volume was completed to the mark with chloroform. The procedure under linearity for the picric acid method was followed. The concentration of CINN was calculated from the regression equation.

Spectrofluorimetric method:

The volume of the laboratory prepared mixtures was completed to the mark with methanol / 0.05 N H_2SO_4 mixture (1:1) and the procedure under linearity for the spectrofluorimetric method was followed. The concentration of CINN was calculated from the regression equation.

- Analysis of pharmaceutical formulations:

Twenty tablets or the contents of twenty capsules were weighed, powdered and thoroughly mixed:

Spectrophotometric methods:

Analysis of pharmaceutical preparations containing CINN

Picric acid method

A quantity of the powder equivalent to 25 mg of CINN was weighed in a 25 ml volumetric flask and 20 ml of chloroform were added. The mixture was sonicated for 15 min, then cooled, diluted to volume with the same solvent and filtered. The first 5 ml of the filtrate was rejected. An aliquot of the filtrate (10ml) was diluted in a 100 ml volumetric flask with the same solvent to obtain a concentration of 100 µg ml^{-1} CINN. This solution was analyzed using the picric acid method as described under linearity.

Analysis of pharmaceutical preparation containing FLUN

A quantity of the powder equivalent to 50 mg of FLUN base was accurately weighed, sonicated with 100 ml water for 30 min and then cooled. The solution was quantitatively filtered into a separatory funnel, alkalinized with 10 ml 1N NaOH solution and extracted with 5 successive 10 ml potions of chloroform. The chloroformic extracts were dried over anhydrous sodium sulphate, collected into a 50 ml volumetric flask and completed to volume with chloroform to give a solution of 1 mg ml^{-1} FLUN base (solution A).

Picric acid method

An aliquot of the obtained solution A (10 ml) was transferred into a 100 ml volumetric flask, diluted with chloroform to obtain a concentration of 100 µg ml^{-1} FLUN. This solution was analyzed by the picric acid method as detailed under linearity.

DDQ method

An aliquot of solution A (25ml) was evaporated to dryness on a water bath. The residue was then dissolved and diluted with methanol in a 100 ml volumetric flask to obtain a concentration of 250 µg ml^{-1} FLUN. The obtained solution was analyzed using the DDQ method as detailed under linearity.

Spectrofluorimetric method:

A quantity of the powder equivalent to 10 mg of CINN or FLUN was weighed each in a 100 ml volumetric flask. 70 ml of methanol / 0.05 N H_2SO_4 mixture (1:1) were added. The mixture was sonicated for 15 min, then cooled, diluted to volume with the same solvent and filtered. The first 10 ml of the filtrate was rejected. An aliquot of the filtrate (10ml) was diluted in a 100 ml volumetric flask with the same solvent, so as to contain 10 µg ml^{-1} of each drug. This solution was analyzed as described under linearity.

RESULTS AND DISCUSSION

In this work, two selective calcium channel blockers, CINN and FLUN were determined using simple spectrophotometric and spectrofluorimetric procedures.

Spectrophotometric methods:

Aiming to rapidness, simplicity and low cost two spectrophotometric methods based upon measuring the absorbance of the colored ion pairs obtained by the reaction of CINN and FLUN with picric acid in chloroform at λ = 402.8 nm as well as that of the colored charge transfer complex of FLUN with DDQ in methanol at λ = 460 nm were suggested.

Picric acid method

Polynitrophenols form with electron donor molecules, especially the amine derivatives charge transfer and proton transfer complexes[38-43]. Picric acid, a polynitrophenol was used for the determination of some amine derivatives through formation of intense yellow colored ion pair complex [41-43]. Interestingly, application of picric acid for quantitative estimation of Orphendrine citrate and Phentolamine mesylate injections is officially in the USP [43].

Because both CINN and FLUN have an aliphatic tertiary amine in their molecular structure with the availability of non-bonding electron donors, they were subjected in the present work to react with picric acid in chloroform. The produced intense yellow colored ion pair complexes were spectrophotometrically measured at λ = 402.8 nm Fig (1,2). Both chloroform and methylene chloride can be used as a solvent, but chloroform is preferred due to its lower volatility. However, the colored ion pair complex was found quite stable even when kept overnight as reported [42]. It is noteworthy, that using of CINN-picric acid reaction product as a gravimetrically reference was stated [9].

The optimum parameters were studied to obtain maximum color development. A volume of 2 ml of 0.025% w/v solution of picric acid in chloroform was found to give maximum color formation in the total volume of 10 ml. The maximum color was developed within 5 minutes at room temperature (25°C) and was found quite stable overnight.

In order to assess stoichiometry of the reaction, Job`s method of continuous variation was applied. The results obtained revealed that the two investigated drugs react with picric acid in a ratio 1:1 under optimum experimental conditions as shown in Fig (4). Beer`s law was obeyed between the absorbance of the colored product at λ = 402.8 nm and the corresponding concentrations in the range of 12-60 µg ml^{-1} and 12-65 µg ml^{-1} for CINN and FLUN, respectively. The regression equations were also computed table I.

Satisfactory results were obtained by applying the proposed picric acid method for the analysis of different concentrations of pure CINN and FLUN within the linearity ranges as summarized in table II.

Besides, the proposed picric acid method was valid for the determination of CINN in laboratory prepared mixtures containing Piracetam with a mean percentage recovery of 100.21 ± 0.65% as represented in table IV.

The suggested picric acid spectrophotometric method was applied to pharmaceutical formulations for analysis of CINN and FLUN, and its validity was further assessed by applying the standard addition technique tables V, VI. The results obtained indicate that additives or other active ingredients present with the studied drugs did not interfere.

DDQ method

DDQ was reported to react as **π**- acceptor (A) with several drugs as **n**-donors ($D^{\cdot}$) to give a highly colored radical anion as a major chromogen (A^{-}) according to the equation:

$$\underset{\text{n-donor}}{D} + \underset{\pi\text{- acceptor}}{A} \longrightarrow \underset{\text{complex}}{[\,D^{\cdot} \text{ --- } A\,]} \longrightarrow \underset{\text{radical ions}}{D^{+} + A^{-}}$$

The above reaction was found to be preferred in polar solvents such as methanol [8,44, 45].

Interestingly, a spectrophotometric method for the determination of CINN using DDQ as analytical reagent was reported [8]. Similarly, the present work describes the use of DDQ for spectrophotometric determination of FLUN. The formed charge transfer complex between FLUN and DDQ exhibits maximum absorbance at λ = 460 nm Fig (3).

Studying of optimum parameters for maximum color formation revealed that a volume of 2 ml of 0.1% w/v solution of DDQ in methanol was found

enough in the total volume of 10 ml. Maximum absorbance readings of the colored product were obtained after 10 minutes at room temperature (25°C) and its stability lasted for further 30 minutes.

The molar ratio determined according to Job`s method of continuous variation indicated of 1:1 ratio for FLUN with DDQ under optimum experimental conditions as shown in Fig (4).

Obedience to Beer`s law was obtained between the measured absorbance of the colored product at λ = 460 nm and concentration in the range of 30-175 µg ml^{-1} for FLUN. The respective regression equation was computed table I.

Replicate determination of different concentrations of pure FLUN within the linearity range was carried out. The concentrations were then calculated from the respective regression equation. Results are given in table II.

Application of the proposed method to capsules for analysis of FLUN gave satisfactory results. The validity of the proposed DDQ spectrophotometric method was further assessed using the standard addition technique table VI.

Spectrofluorimetric method:

Spectrofluorimetric measurements are usually used because of their excellent sensitivity and more selectivity in comparison with other methods of analysis.

It was reported that most fluorescent organic compounds are those have **π**-system within their chemical structure such as polyene and aromatic derivatives. The fluorescence efficiencies are greatest when the **π**-system is rigid and planner in both ground and excited states[46]. Besides, compounds of diphenylmethane chromophore are known to have native fluorescence, when properly excited [47].

Accordingly, the ability of both CINN and FLUN to produce native fluorescence in methanol / 0.05 N H_2SO_4 mixture (1:1) at λ_{em} = 305 nm, 298 nm when excited at λ_{ex} =264 nm, 254 nm, respectively is more or less logical Fig (5,6). This may be attributed to their cinnamyl and diphenylmethane chromophores.

Optimum conditions to obtain maximum relative fluorescence intensity were studied. The composition of the solvent system was adjusted after varying type of alcohol, acid; normality of the acid and alcohol to acid ratio. Maximum relative fluorescence intensities were achieved on using at room temperature (25°C) methanol / 0.05 N H_2SO_4 (1:1) mixture as a solvent.

Linear correlation was obtained between the measured relative fluorescence intensities and the corresponding concentrations in the range of 0.2- 4 µg ml^{-1} and 0.2- 5 µg ml^{-1} for CINN and FLUN, respectively. The respective regression equations were computed table I.

To check the reproducibility of the results, the proposed method was successfully applied for replicate determination of different concentrations of CINN and FLUN in their bulk powders. The obtained results showed good accuracy and precision table III. In addition, the proposed method was valid for the determination of CINN in laboratory prepared mixtures containing Piracetam with a mean percentage recovery of 99.92 + 0.76% as represented in table IV.

The suggested spectrofluorimetric method was applied to pharmaceutical formulations for analysis of CINN and FLUN, and its validity was further assessed by applying the standard addition technique tables V, VI. The results obtained indicate that additives or other active ingredients present with the studied drugs did not interfere.

Conclusion

The characteristics of the proposed methods are summarized in table VII.

The results of the proposed methods were statistically compared with those obtained by adopting the comparison methods (for CINN, official titrimetric method B. P. 2001 [2]; for FLUN, D_1 spectrophotometric method in methanol [34]). The t-and F-values were calculated and found to be less than the tabulated ones indicating no significant difference with respect to accuracy and precision table VIII.

Furthermore, statistical analysis of the results obtained by the proposed methods has been carried out by SPSS statistical package version 11 using one way ANOVA (F-test) followed by Dunnett (t-test) at $P < 0.05$. The test ascertains that the proposed methods to be as precise and accurate as the reference methods table IX**.**

The results obtained indicate that the proposed methods may be classed amongst rapid and sensitive procedures. In terms of simplicity and expense the proposed methods can be considered satisfactory in comparison with others. These merits, suggest the use of the proposed methods in routine and quality control laboratories for these two drugs.

Table I: Analytical parameters for the determination of CINN and FLUN by the proposed methods

Method	Compound Determined	λ of measurement	Regression equation	Correlation coefficient(r)	Linearity range μg ml^{-1}
Spectrophotometric methods:					
. picric acid method	CINN	402.8nm	A*= 0.0189 C*- 0.0419	0.9993	12 - 60
	FLUN	402.8nm	A*= 0.0175 C*- 0.0491	0.9997	12 - 65
. DDQ method	FLUN	460.0nm	A*= 0.0054 C*+ 0.0011	0.9995	30 - 175
Spectrofluorimetric method	CINN	305.0nm	F*= 4.7650 C*+ 0.4747	0.9999	0.2 - 4
	FLUN	298.0nm	F*= 2.5227 C*+ 0.4812	0.9994	0.2 - 5

* **A** is the absorbance, **F** is the relative fluorescence intensity and **C** is the concentration (μg ml^{-1}).

Table II: Determination of pure samples of CINN and FLUN by the proposed spectrophotometric methods

	CINN			**FLUN**					
	Picric acid method			**Picric acid method**			**DDQ method**		
Ex No.	Taken μg ml^{-1}	Found* μg ml^{-1}	% Recovery	Taken μg ml^{-1}	Found* μg ml^{-1}	% Recovery	Taken μg ml^{-1}	Found* μg ml^{-1}	% Recovery
1	14	13.857	98.98	15	14.920	99.47	40	40.537	101.34
2	18	17.825	99.03	20	19.834	99.17	60	60.722	101.20
3	24	24.016	100.07	27	27.263	100.97	80	79.981	99.98
4	32	31.847	99.52	35	34.691	99.12	100	99.796	99.80
5	40	40.312	100.78	40	40.406	101.02	120	118.500	98.75
6	45	45.180	100.40	48	48.749	101.56	135	134.056	99.30
7	52	52.322	100.62	55	55.149	100.27	150	151.833	101.22
8	58	58.143	100.25	63	62.806	99.69	170	169.056	99.44
Mean ± RSD%		**99.96 ± 0.70**			**100.16 ± 0.94**			**100.13 ± 1.00**	

*Each result is the average of three experiments.

Table III: Determination of pure samples of CINN and FLUN by the proposed spectrofluorimetric method

	CINN			**FLUN**		
Ex No.	Taken μg ml^{-1}	Found* μg ml^{-1}	% Recovery	Taken μg ml^{-1}	Found* μg ml^{-1}	% Recovery
1	0.2	0.202	101.00	0.2	0.198	99.00
2	0.8	0.803	100.38	0.5	0.496	99.20
3	1.2	1.191	99.25	0.8	0.800	100.00
4	1.5	1.516	101.07	1.5	1.494	99.60
5	2.2	2.203	100.14	2.5	2.525	101.00
6	2.8	2.817	100.61	3.5	3.476	99.31
7	3.5	3.485	99.56	4.2	4.229	100.69
8	3.8	3.777	99.39	4.8	4.764	99.25
Mean ± RSD%		**100.18 ± 0.71**				**99.76 ± 0.74**

*Each result is the average of three experiments.

Table IV: Determination of CINN in laboratory prepared mixtures (with Piracetam) **by the proposed methods**

Spectrophotometric (Picric acid) method					Spectrofluorimetric method				
EX No.	Taken (μg ml^{-1}) **CINN**	Piracetam	Found* (μg ml^{-1}) **CINN**	% Recovery	Ex No.	Taken (μg ml^{-1}) **CINN**	Piracetam	Found*(μg ml^{-1}) **CINN**	% Recovery
1	12	192	12.010	100.08	1	0.2	3.2	0.199	99.50
2	18	288	17.996	99.98	2	1.0	16.0	0.989	98.90
3	24	384	24.086	100.36	3	1.5	24.0	1.511	100.73
4	30	480	30.252	100.84	4	2.0	32.0	2.013	100.65
5	36	576	35.722	99.23	5	2.5	40.0	2.489	99.56
6	45	720	44.771	99.49	6	3.0	48.0	2.976	99.20
7	52	832	52.288	100.55	7	3.5	56.0	3.532	100.91
8	60	960	60.684	101.14	8	4.0	64.0	3.995	99.88
Mean±RSD%			**100.21 ± 0.65**					**99.92 ± 0.76**	

*Each result is the average of three experiments.

Table V: Determination of CINN in Pharmaceutical formulations by the proposed methods and application of standard addition technique

Ex No	Spectrophotometric (Picric acid) method						Spectrofluorimetric method					
	Claimed ($\mu g\ ml^{-1}$)	Found*	% Recovery	Added ($\mu g\ ml^{-1}$)	Found*	% Recovery	Claimed ($\mu g\ ml^{-1}$)	Found*	% Recovery	Added ($\mu g\ ml^{-1}$)	Found*	% Recovery
- Stugeron tablets [R]							**- Stugeron tablets [R]**					
1	14	13.910	99.36	30	30.212	100.71	0.2	0.198	99.00	3.0	3.029	100.97
2	18	17.772	98.73	18	18.043	100.24	0.8	0.803	100.38	2.0	2.014	100.70
3	18	17.772	98.73	40	39.524	98.81	1.2	1.202	100.17	1.2	1.196	99.67
4	24	23.963	99.85	24	23.862	99.43	1.2	1.202	100.17	2.4	2.381	99.21
5	24	23.963	99.85	35	34.709	99.17	1.8	1.789	99.39	1.8	1.805	100.28
6	32	32.005	100.02	20	19.788	98.94	2.4	2.377	99.04	1.4	1.385	98.93
7	40	39.571	98.93	15	15.080	100.53	3.0	2.964	98.80	1.0	0.993	99.30
8	45	44.598	99.11	12	11.905	99.21	3.5	3.500	100.00	0.5	0.503	100.60
Mean ± RSD%		**99.33 ±0.51**			**99.63 ± 0.75**		**Mean ± RSD%**		**99.54 ±0.64**		**99.96 ± 0.78**	
- Cinartam capsules [R]							**- Cinartam capsules [R]**					
1	14	13.751	98.22	30	30.159	100.53	0.2	0.194	97.00	3.0	3.022	100.73
2	18	17.561	97.56	18	17.883	99.35	0.8	0.792	99.00	2.0	2.004	100.20
3	18	17.561	97.56	40	40.159	100.40	1.2	1.170	97.50	1.2	1.213	101.08
4	24	23.487	97.86	24	23.756	98.98	1.2	1.170	97.50	2.4	2.382	99.25
5	24	23.487	97.86	35	35.079	100.23	1.8	1.762	98.22	1.8	1.780	98.89
6	32	31.265	97.70	20	20.105	100.53	2.4	2.335	97.29	1.4	1.400	100.00
7	40	39.519	98.80	15	14.973	99.82	3.0	2.912	97.07	1.0	1.003	100.30
8	45	44.598	99.11	12	11.852	98.77	3.5	3.416	97.60	0.5	0.493	98.60
Mean ± RSD%		**98.21 ± 0.63**			**99.83 ± 0.71**		**Mean ± RSD%**		**97.67 ± 0.71**		**99.88 ± 0.88**	

*Each result is the average of three experiments.

Table VI: Determination of FLUN in Pharmaceutical formulation by the proposed methods and application of standard addition technique

Ex No.	Spectrophotometric methods												Ex No.	Spectrofluorimetric method					
	Picric acid method						DDQ method												
	Claimed ($\mu g\ ml^{-1}$)	Found*	% Recovery	Added ($\mu g\ ml^{-1}$)	Found*	% Recovery	Claimed ($\mu g\ ml^{-1}$)	Found*	% Recovery	Added ($\mu g\ ml^{-1}$)	Found*	% Recovery		Claimed ($\mu g\ ml^{-1}$)	Found*	% Recovery	Added ($\mu g\ ml^{-1}$)	Found*	% Recovery
1	15	14.691	97.94	15	14.858	99.05	35	34.611	98.89	140	140.185	100.13	1	0.2	0.199	99.50	4.5	4.482	99.60
2	**15**	**14.691**	**97.94**	**50**	**50.400**	**100.80**	**40**	**39.611**	**99.03**	**40**	**40.371**	**100.93**	2	0.8	0.794	99.25	4.0	4.020	100.50
3	20	19.434	97.17	20	20.200	101.00	60	58.658	97.81	60	59.629	99.38	3	1.6	1.573	98.31	1.6	1.593	99.56
4	20	19.434	97.17	40	39.772	99.43	70	69.056	98.65	70	70.185	100.26	4	1.6	1.573	98.31	3.0	3.002	100.07
5	30	29.320	97.73	30	30.040	100.13	80	78.870	97.59	80	80.832	101.04	5	2.4	2.393	99.71	2.4	2.414	100.58
6	40	39.608	99.02	18	17.911	98.51	100	97.089	97.09	50	49.444	98.89	6	2.4	2.393	99.71	2.0	1.971	98.55
7	45	44.797	99.55	15	15.093	100.62	125	123.315	98.65	30	30.185	100.62	7	3.2	3.159	98.72	1.3	1.288	99.08
8	50	49.434	98.87	12	11.905	99.21	140	137.944	98.53	35	34.630	98.94	8	4.2	4.097	97.54	0.8	0.805	100.63
Mean ± RSD%			**98.38 ± 0.92**			**99.84 ± 0.92**			**98.28 ± 0.71**			**100.02 ± 0.86**	**Mean± RSD%**			**98.84 ± 0.83**			**99.82 ± 0.76**

*Each result is the average of three experiments.

Table VII: Statistical data of the results obtained by applying the proposed methods for the determination of CINN and FLUN

Data	CINN		FLUN		
	Spectrophotometric (picric acid method)	Spectrofluorimetric method	Spectrophotometric methods (picric acid)	(DDQ)	Spectrofluorimetric method
Intercept	0.0419	0.4747	0.0491	0.0011	0.4812
S_a*	0.0090	0.0060	0.0070	0.0070	0.0090
Slope	0.0189	4.7650	0.0175	0.0054	2.5227
S_b**	0.0010	0.0030	0.0010	0.0010	0.0040
Mean Of (y)	0.6050	9.0510	0.6080	0.5510	5.5890
S.E.(y) = S.D.(y)/$N^{1/2}$	0.0990	0.2880	0.1210	0.0970	0.1610

* Standard error of intercept.
** Standard error of slope.

Table VIII: Statistical comparison between the results of the proposed methods and reference methods in determination pure samples

	CINN			FLUN			
Value	The proposed			The proposed			
	Spectrophotometric (picric acid method)	Spectrofluorimetric method	Official method**	Spectrophotometric methods (picric acid)	(DDQ)	Spectrofluorimetric method	reference method***
Mean	99.96	100.18	99.84	100.16	100.13	99.76	100.07
S.D.	0.70	0.71	0.71	0.94	1.00	0.74	0.57
n	8	8	6	8	8	8	7
Variance	0.490	0.504	0.504	0.884	1.000	0.548	0.325
t	0.313	0.888	(2.179)*	0.230	0.147	0.928	(2.160)*
F	0.972	1.000	(4.880)*	2.720	3.078	1.685	(4.210)*

* Figures between parentheses represent corresponding tabulated values of t and F at P=0.05
** A non aqueous titrimetric method [2].
*** A first derivative spectrophotometric method [34].

Table IX: Statistical analysis of the results obtained by applying the proposed methods for the determination of CINN and FLUN as compared to those of the reference methods:

Method	CINN		FLUN	
	N	Mean + RSD%	N	Mean + RSD%
Spectrophotometric methods:				
. Picric acid	8	99.956 + 0.699	8	100.159 + 0.934
. DDQ	--	-----	8	100.129 + 0.999
Spectrofluorimetric method	8	100.175 + 0.712	8	99.756 + 0.742
Reference method*.	6	99.843 + 0.713	7	100.070 + 0.568
F- value		0.406		0.391
P- value		0.672		0.760

-There is no significant difference between the methods using one way ANOVA (F- test) Followed by Dunnett t- test at $P < 0.05$.
-Dunnett t- test: treat one group as control (Reference method) and compare all other groups against it.
*Reference methods: - B.P. (2001) a titrimetric method for CINN [2].
- D_1 spectrophotometric method for FLUN [34].

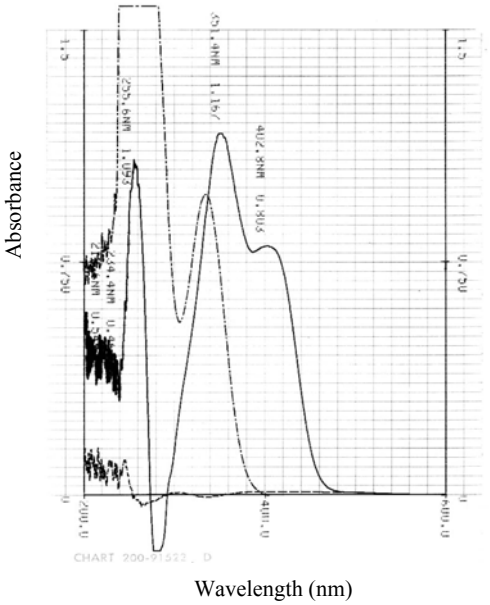


Fig 1: Absorption spectra of:
a. 45 µg ml^{-1} CINN + 2 ml 0.025% picric acid (—) in chloroform.
b. 720 µg ml^{-1} Piracetam + 2 ml 0.025% picric acid (---) in chloroform.
c. Reagent blank 2 ml 0.025% picric acid (— -) in chloroform.

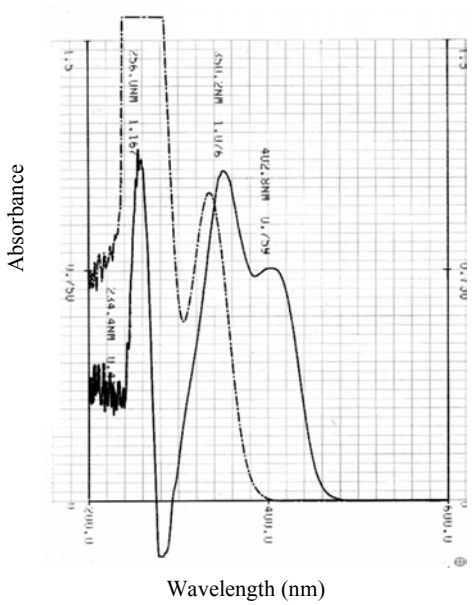


Fig 2: Absorption spectra of:
a. 45 µg ml^{-1} FLUN + 2 ml 0.025% picric acid (—) in chloroform.
b. Reagent blank 2 ml 0.025% picric acid (— -) in chloroform.

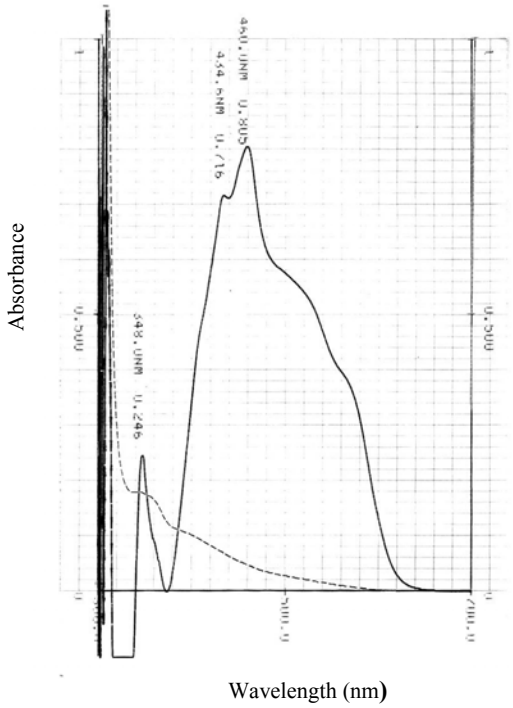


Fig 3: Absorption spectra of:
a. 150 µg ml^{-1} FLUN + 2 ml 0.1% DDQ (—) in methanol.
b. Reagent blank 2 ml 0.1% DDQ (---) in methanol.

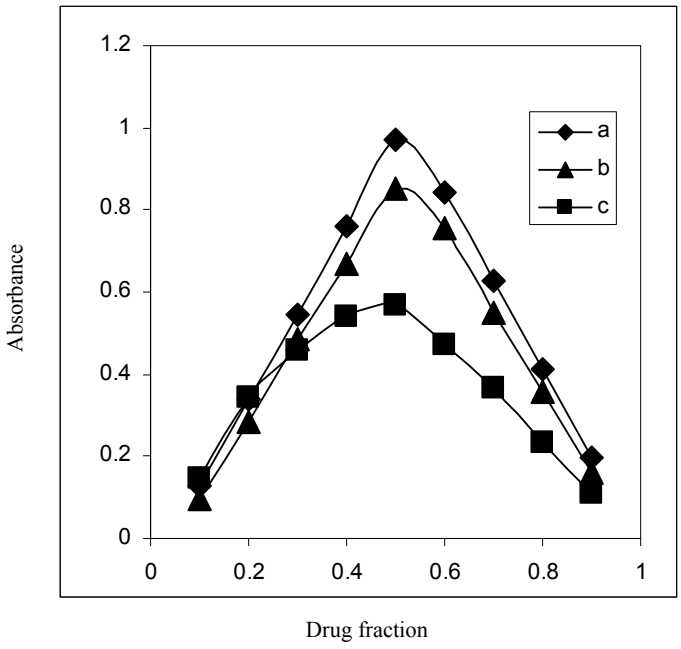


Fig 4: Continuous variation method applied for complex formed:
a. CINN with picric acid.
b. FLUN with picric acid.
c. FLUN with DDQ.

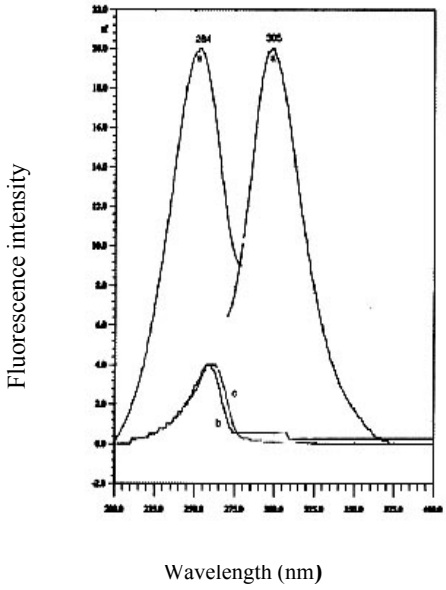


Fig 5: Excitation and emission spectra of:
a. CINN (4 µg ml^{-1}).
b. Piracetam (64 µg ml^{-1}).
c. Blank: methanol/ 0.05N H_2SO_4 (1:1).

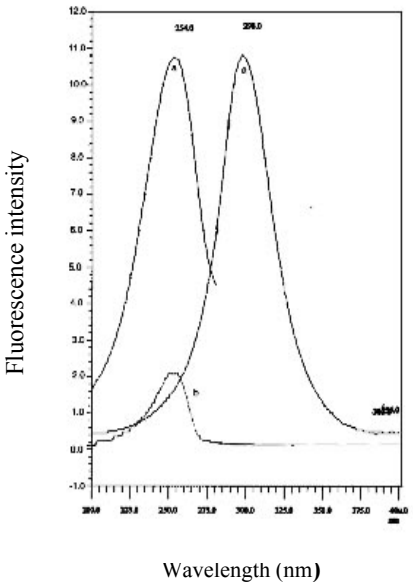


Fig 6: Excitation and emission spectra of
a. FLUN (4 µg ml^{-1}).
b. Blank: methanol/ 0.05N H_2SO_4 (1:1).

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طرق طيفضوئية ولصفية لتقديركلا من السيناريزين وثنائى أيدروكلوريد الفلونارىزين فى الصورة النقية والمستحضرا ت الصيدلية.

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تم فى هذا البحث تعيين إثنين من مثبطات مستقبل الهستامين هـ١ وهما السيناريزين وثنائى أيدروكلوريد الفلونارىزين بطرق طيفضوئية و لصفية بسيطة و سريعة.

وقد إعتمدت الطرق الطيفضوئية على تفاعل كل من العقارين المذكورين مع حمض البكريك وقياس الإمتصاص الطيفى لنواتج التفاعل الملونة عند طول موجة ٤٠٢,٨نم فى حدود تركيزات تتروا ح بين ١٢- ٦٠ ؛ ١٢- ٦٥ ميكروجرام/ مل وبدقة بلغت ٩٩,٩٦ ± ٠,٧٠ ؛ ١٠٠,١٦ ± ٠,٩٤لكل من السيناريزين والفلونارىزين فى الصورة النقية على التوالى. وكذلك تم تعيين عقارثنائى أيدروكلوريد الفلونارىزين إعتمادا على قياس الإمتصاص الطيفى لمعقد إنتقال الشحنة الملون الناتج من تفاعله مع ٢و٣- ثنائى كلورو- ٥و٦- ثنائى سيانو- بارا- بنزوكينون عند طول موجة ٤٦٠نم فى حدود تركيز يتروا ح بين٣٠- ١٧٥ ميكروجرام/ مل وبدقة بلغت ١٠٠,١٣ ± ١,٠٠.

هذا وقد تم تقديركل من العقارين بطريقة لصفية بإستثارتهما عند طول موجة ٢٦٤؛ ٢٥٤نم وقياس الوميض اللصفى الطبيعى الناتج عند طول موجة ٣٠٥ ؛ ٢٩٨ نم فى حدود تركيزات تتروا ح بين٠,٢- ٤ ؛ ٠,٢- ٥ ميكروجرام/ مل وبدقة بلغت ١٠٠,١٨ ± ٠,٧١ ؛ ٩٩,٧٦ ± ٠,٧٤ لكل من السيناريزين والفلونارىزين فى الصورة النقية على التوالى.

وقد طبقت الطرق المقترحة بنجاح فى تحليل العقارين المذكورين فى المستحضرات الصيدلية المحتوية عليهما دون أى تداخل سواءا من الاضافات الصيدلية أو مركب البيراسيتام حال تواجده مع السينارزين. هذ ا وقد قورنت النتائج التى امكن الحصول عليها احصائيا مع الطرق المرجعية المختارة ولم تكن هناك اية فروق احصائية.