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Spectrophotometric Determination of Aciclovir, Cefepime Hydrochloride, Etamsylate and Metoclopramide Hydrochloride Using 1,10 Phenanthroline – Fe(III) Reagent

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Abstract

A simple, rapid,sensitive and accurate method for the determination of aciclovir,cefepime HCl, etamsylate and metoclopramide HCl in pure form and in pharmaceutical formulations is developed.The method is based on the formation of tris(o-phenanthroline) iron(II) complex (Ferroin) upon the reaction of the cited drugs with iron(III)-o- phenanthroline mixture.The ferroin complex is colorimetrically measured at λ_{max} 510 nm against a reagent blank.Optimization of the experimental conditions is described.Beer's law is obeyed in the concentration range from 0.25-30 μg ml^{-1} with molar absorpitivities (ε) ranging from 4.796×10^3 –9.512×10^4 $L.mol^{-1}.cm^{-1}$ and Sandell sensitivities (S) of 2.129×10^{-3} – 34.5×10^{-3} μg cm^{-2}.The developed method is applied successfully for the determination of the cited drugs in pure forms and in the corresponding pharmaceutical formulations without any interferences from common excipients.

Keywords:Spectrophotometry,1,10 - Phenanthroline – Fe(III) reagent, Pure forms,Pharmaceutical formulations.

INTRODUCTION

Aciclovir is an anti-viral agent that is used currently for treatment of Herps Simplex[1].It has been determined by several procedures e.g. spectrophotometry[2,3],HPLC[4-7] and radio-immuno-assay[8].

Cefepime hydrochloride is a fourth generation cephalosporin that's widely used as anti-bacterial and administred via injection[1].It has been determined by several procedures e.g. spectrophotometry[9],diffuse reflectance IR& X-ray diffraction[10] and HPLC[11].

Etamsylate is a haemostatic agent which is used for prophylaxis and control of hemorrhage from small blood vessels[1].Several procedures were reported for its analysis e.g. spectrophotometry[12,13], HPLC[14],voltammetry[15],polarography[16,17] and potentiometry[18].

Metoclopramide hydrochloride,an antiemetic procaine derivative that's used in gastro-intestinal diagnostics(GI) and in treatment of GI disorders[1].Some methods that have been reported for its determination include spectrophotometry[19-25],NMR[26] and HPLC[27].

The aim of the present work is to apply 1,10-phenanthroline – Fe^{3+} reagent to react with the studied drugs yielding a colored ferroin complex and presenting a simple and rapid assay procedure for the studied drugs in pure form and in pharmaceutical formulations.This work describes a spectrophotometric method that can be used in laboratories where modern and expensive apparatus such as that required for GLC or HPLC is not available.

EXPERIMENTAL

Apparatus:

* Shimadzu UV 260 recording spectrophotometer with matched 10 mm quartz cells was used for all absorbance measurements.

Materials and Reagents :

All chemicals used were of analytical grade and all solutions were freshly prepared in doubly distilled water.

1- Aciclovir;Memphis Co. for Pharm.&Chem. Ind.,OM lab. Ltd., Meyrin,Geneva,Switzerland.

2- Cefepime hydrochloride;Bristol Myers Squibb,Egypt,A Bristol Myers Squibb company,New-York .

3- Etamsylate;Memphis Co. for Pharm. & Chem. Ind.,OM lab. Ltd., Meyrin,Geneva,Switzerland.

4- Metoclopramide hydrochloride;Alexandria Co. for Pharmaceuticals , Egypt.

5- Preparation of iron(III)-o-phenanthroline reagent[28]: mix 0.198 g of 1,10-phenanthroline monohydrate (Winlab,U.K.) with 2 ml of 1M HCl and 0.160 g of ferric ammonium sulphate dodecahydrate (Fluka,Switzerland) and dilute with water to 100 ml.The reagent is stable for six weeks if stored in a dark bottle and away from light.

6- Standard drug solutions:prepared by dissolving 100 mg of pure drug sample in 100 ml distilled water (heating for 2 minutes in boiling water bath is applied in case of aciclovir) to obtain working standard solution of concentration 1 mg ml^{-1}.

Formulations :

The following commercial formulations were subjected to the analytical procedures :-

1- Novirus®capsules:Glaxowellcome Egypt,Cairo,A.R.E.,(200 mg aciclovir per capsule).

2- Zovirax®vials:The Wellcome foundation Ltd.,London (250 mg aciclovir Na per vial).

3- Maxipem®vials:Bristol Myers Squibb,Egypt,(500 mg cefepime hydrochloride per vial) .

4- Dicynone®tablets:Memphis,OM lab. Ltd.,Switzerland,(250 mg etamsylate per tablet).

5- Haemostop®tablets:Amoun Pharm. Co.,Egypt,(250 mg etamsylate per tablet).

6- Primperan® tablets:Memphis Co.,Delagrange,Paris,(10 mg metoclopramide HCl per tablet).

7- Meclopram®ampoules:Alexandria Co. for Pharmaceuticals, Egypt,(10 mg metoclopramide HCl per ampoule).

Procedure:

For pure pharmaceuticals :

Aliquots of the standard drug solutions ranging from 2.5-300 μg ml^{-1} (Tab.2) were pipetted into test tubes,followed by 1.5- 2.5 ml Fe(III)-o- phenanthroline reagent,the tubes were then heated on boiling water bath for the specified times (Tab.1).After completion of heat treatment the solution was cooled to room temperature,transferred carefully and quantitatively to 10 ml volumetric flasks and the volume was made up to the mark with distilled water.The developed colored complex was measured at 510 nm against a reagent blank similarly treated (drug is omitted).The concentration is then calculated from regression equations or calibration graphs.

For dosage forms:

For tablets:-

Ten tablets were finely ground.A portion of powder equivalent to 100 mg of the active ingredient was extracted with 3 x 25 ml portions of distilled water,the portions were filtered, the filter washed with distilled water and the solution diluted to 100 ml with distilled water.The drug content in the obtained extract was determined as described under the general procedure.

For capsules :

The contents of ten capsules were emptied.A portion equivalent to 100 mg of the active ingredient was extracted with 3 x 25 ml portions distilled water by aid of heating for 2 min. in boiling water bath and the portions were filtered into 100 ml calibrated flask,complete as described under tablets and follow the general procedure.

For vials :

The contents of 2 vials were mixed and an amount of aciclovir Na equivalent to 100 mg of aciclovir was weighed and dissolved in 100 ml distilled water,complete following the general procedure.

For ampoules:

The requisite volume equivalent to 100 mg drug was transferred into 100 ml volumetric flask and the volume was made up to the mark with water then complete as before following the general procedure.

Tab.1. Analytical data for the determination of examined drugs

Drug	λ_{max} (nm)	Reagent volume (ml)	Heating time (min.)	Stability (hrs)
1- Aciclovir	510	2.5	30	24
2- Cefepime HCl	510	1.5	25	24
3- Etamsylate	510	2	20	24
4- Metoclopramide HCl	510	2	20	24

Tab.2. Optical characteristics for the reaction of examined drugs with Iron(III)-o-phenanthroline reagent,"Calibration data".

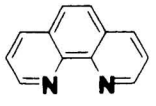
Drug	Detection range (μg ml^{-1})	Molar absorpitivity ($L.mol^{-1}cm^{-1}$)	Sandell sensitivity (μg cm^{-2})	r	b	A
1-Aciclovir	5-30	4.796×10^3	2.129×10^{-3}	0.99999	0.0204	0.0108
2-Cefepime HCl	0.45-4.2	9.512×10^4	18.4×10^{-3}	0.99999	0.175	0.0114
3-Etamsylate	0.25-2.75	9.08×10^4	34.5×10^{-3}	0.99999	0.338	4.746×10^{-3}
4-Metoclopramide HCl	2-11	1.977×10^4	5.58×10^{-3}	0.99999	0.0543	7.255×10^{-3}

**Regression equation* :- A=a+bc

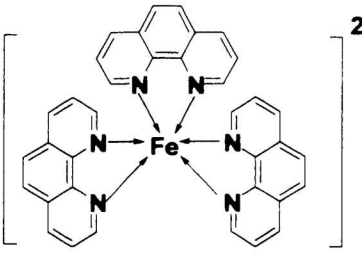
a = intercept b = slope c = concentration (μg ml^{-1}) A = absorbance unit

Results and Discussion

1,10 – phenanthroline is an organic base which contains iron(II) specific group[29].



1,10 - phenanthroline



Ferroin

[Iron(II)-o-phenanthroline complex]

The proposed method is based on the formation of tris(o- phenanthroline) iron(II) chelate upon the reaction of cited drugs with an iron(III)-o-phenanthroline reagent.The reaction proceeds through reduction of iron(III) ions to iron(II) and subsequent formation of intensive orange - red coloration of the complex.

The absorption spectra of the colored species in the proposed method shows a characteristic λ_{max} at 510 nm where the orange-red color was completely developed.The experimental conditions were established by varying each parameter individually[30] in order to establish the favorable experimental conditions.

For the proposed method,the cited drugs were allowed to react with Fe(III) in the presence of 1,10-phenanthroline and factors as temperature,reaction time and reagent concentration were studied.

Samples prepared as described under the general procedures were left at different sets of temperatures ranging from 25 - 100°C for a fixed time (Tab.1),(Fig.1),maximum absorbance and sensitivity were obtained by heating the reactants in boiling water bath.

At lower temperatures the rate of color development becomes progressively slower and it proceeds very slowly at room temperature where there was no visible formation of any colored compound at room temperature.

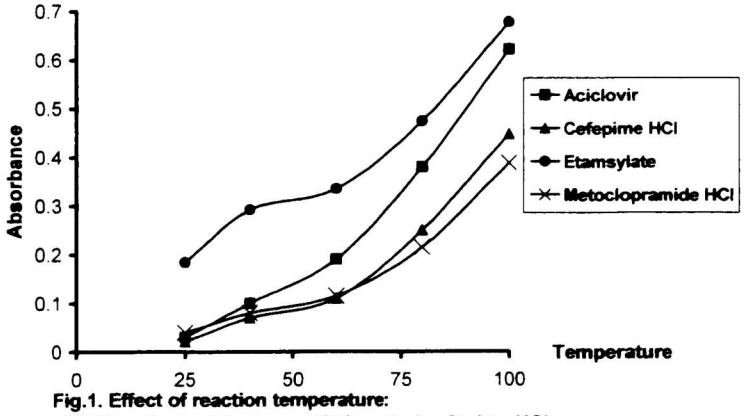


Fig.1. Effect of reaction temperature:
(1) 30 µg / ml aciclovir (2) 3 µg / ml cefepime HCl
(3) 2 µg /ml etamsylate (4) 7 µg /ml metoclopramide HCl

On the other hand,heating times on boiling water bath were varied and the absorbance was measured.It was found that 20 minutes were sufficient for metoclopramide hydrochloride and etamsylate,25 minutes for cefepime hydrochloride while aciclovir required 30 minutes for complete color development (Tab.1).Further heating causes no change in the color while measurements taken before the recommended times render inaccurate results so heating in boiling water bath for the recommended times was adopted for all subsequent measurements.

Another study on the volume of iron(III)-o-phenanthroline reagent was done where various volumes ranging from 0.5 – 3.5 ml were used. It was found that 1.5 - 2.5 ml were adequate for maximum absorbance and sensitivity (Tab.1).Smaller volumes gave incomplete complex formation,while larger volumes had no effect on

the complex formation, although absorbance increased slightly due to background of the reagent used. The color obtained was stable for at least 24 hours at room temperature (Tab.1).

The effects of common additives and excepients were studied and it was found that there were no interferences,so the examined drugs can be assayed directly in their dosage forms by the suggested method without prior extraction or separation.

Calibration graphs

Calibration graphs were constructed by plotting the concentration of the drug against the corresponding absorbance values.The resulting graphs were linear up to 30 μg ml^{-1} aciclovir , 4.2 μg ml^{-1} cefepime hydrochloride, 2.75 μg ml^{-1} etamsylate and 11 μg ml^{-1} metoclopramide hydrochloride.

The linearity of calibration graphs is apparent from the correlation coefficient,r, and the intercepts which are nearly close to zero.All calibration data are calculated and listed in (Tab. 2).

Sensitivity, accuracy and precision:

The mean molar absorpitivity **(ε)** and Sandell's sensitivity **(S)** as calculated from Beer's law are presented in (Tab.2).The %S.D. and % range of S.E. at 95% confidence limits are given in (Tab. 3&4).

The utility of this method was verified by means of replicate measurements of pharmaceutical formulations and recovery experiments.Recoveries were determined either by standard addition method or by calibration method.

The performance of this method was assessed by calculation of "t" and F-values compared with the reported methods for determination of these drugs.Results showed that no significant difference in accuracy and precision between the proposed and the reported methods (Tab.3&4).

Tab. 3. Comparative analytical results of the proposed and the reported method for the tested drugs in pure forms

Statisical parameters	Aciclovir		Cefepime HCl		Etamsylate		Metoclopramide HCl	
	Proposed method	Official method[31]	Proposed method	Reference method[9]	Proposed method	Official method[31]	Proposed method	Official method[31]
Mean % recovery	100.01	99.94	99.85	99.81	99.86	100.3	99.91	99.77
N	6	3	10	5	11	4	10	3
Variance	0.043	$7.6X10^{-3}$	0.190	0.059	0.104	0.250	0.159	0.154
S.D.	0.207	0.087	0.436	0.243	0.323	0.500	0.399	0.393
S.E.	0.085	0.050	0.138	0.109	0.097	0.250	0.126	0.227
R.S.D.	0.207	0.087	0.437	0.243	0.323	0.499	0.399	0.394
"t"	0.710(2.365)		0.227(2.160)		1.641(2.160)		0.539(2.201)	
F	5.66 (5.79)		3.22(3.63)		2.404(3.71)		1.032(4.26)	

N = Number of experiments S.D. = Standard Deviation S.E. = Standard Error

R.S.D. = Relative Standard Deviation t = "t" test of unpaired data F = Variance test

Tab.4. Comparative analytical results of the proposed and reported method for the tested drugs in some pharmaceutical formulations.

Formulation	Statistical parameters	Proposed method		Reference method
		Calibration method	Standard addition method	
1- Novirus® capsules	Mean %recovery	99.87	99.92	100.39[31]
	N	7	7	3
	Variance	0.087	0.104	0.120
	S.D.	0.295	0.322	0.346
	S.E.	0.111	0.122	0.200
	R.S.D.	0.295	0.322	0.345
	"t"	2.273(2.306)	2.006(2.306)	
	F	1.379 (5.14)	1.154 (5.14)	
2- Zovirax® vials	Mean %recovery	100.49	100.45	100.3 [31]
	N	6	7	5
	Variance	0.128	0.172	0.084
	S.D.	0.358	0.415	0.290
	S.E.	0.146	0.157	0.130
	R.S.D.	0.356	0.413	0.289
	"t"	0.972 (2.262)	0.736 (2.228)	
	F	1.524 (5.19)	2.048 (4.53)	

Continue **Tab.4.**

Formulation	Statistical Parameters	Proposed method		Reference method
		Calibration method	Standard addition method	
3- Maxipem® vials	Mean %recovery	99.63	99.52	99.8 [9]
	N	9	7	5
	Variance	0.262	0.217	0.081
	S.D.	0.512	0.466	0.284
	S.E.	0.171	0.176	0.127
	R.S.D.	0.514	0.468	0.285
	"t"	0.798 (2.179)	1.290 (2.228)	
	F	3.24 (3.84)	2.679 (4.53)	
4- Dicynone® tablets	Mean %recovery	99.24	98.58	99.38 [31]
	N	6	9	4
	Variance	0.476	0.391	0.608
	S.D.	0.690	0.625	0.780
	S.E.	0.282	0.208	0.390
	R.S.D.	0.695	0.634	0.785
	"t"	0.291 (2.306)	1.810 (2.201)	
	F	1.277 (5.41)	1.555 (4.07)	
5- [illegible]®	Mean %recovery	99.36	99.65	99.9 [31]
	N	6	7	6

Haemostop® tablets	Variance	0.198	0.203	0.476
	S.D.	0.445	0.450	0.690
	S.E.	0.182	0.170	0.282
	R.S.D.	0.448	0.452	0.691
	"t"	1.609 (2.228)	0.759 (2.201)	
	F	2.404 (5.05)	2.345 (4.39)	
6-Primperan® tablets	Mean %recovery	99.09	99.72	99.4 [31]
	N	8	8	5
	Variance	0.338	0.144	0.295
	S.D.	0.581	0.379	0.543
	S.E.	0.205	0.134	0.243
	R.S.D.	0.586	0.380	0.546
	"t"	1.266 (2.201)	1.153 (2.201)	
	F	1.146 (4.12)	2.049 (4.12)	
7-Meclopram® ampoules	Mean %recovery	99.94	99.66	99.7 [31]
	N	9	8	5
	Variance	0.288	0.166	0.160
	S.D.	0.537	0.407	0.400
	S.E.	0.179	0.144	0.179
	R.S.D.	0.537	0.408	0.401
	"t"	0.948(2.179)	0.174 (2.201)	
	F	1.80 (3.84)	1.038 (4.12)	

N = Number of experiments S.D. = Standard Deviation. S.E. = Standard Error. R.S.D.= Relative Standard Deviation. t = "t" test of unpaired data. F = Variance test.

Conclusion

The proposed method is advantageous when compared to many of the reported spectrophotometric methods in having higher sensitivity.The data given before reveal that proposed method is simple, sensitive with good accuracy and precision.It doesn't require expensive toxic chemicals or sophisticated experimental setup.It can be used directly to determine the examined drugs without prior extraction as common additives don't interfere.

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