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Il Farmaco 57 (2002) 865–870

IL FARMACO

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Spectrophotometric determination of acyclovir in some pharmaceutical formulations

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Received 20 March 2002; accepted 29 June 2002

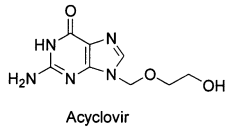
Abstract

A simple and reliable spectrophotometric method has been developed for the determination of acyclovir in pharmaceutical formulations. The method is based on its oxidative coupling reaction with 3-methylbenzothiazolin-2-one hydrazone (MBTH) in the presence of $FeCl_3$ as an oxidant to produce deep-green colored species measurable at 616 nm. The absorbance–concentration plot is linear over the range 20–200 μg ml^{-1} with minimum detectability of 1.06 μg ml^{-1} (4.71×10^{-6} M). The molar absorptivity was 9.41×10^2 l mol^{-1} cm^{-1} with correlation coefficient ($n = 7$) of 0.9998. The different experimental parameters affecting the development and stability of the color were studied carefully and optimized. The proposed method was applied successfully to the determination of acyclovir in its dosage forms. The percentage recoveries ±SD ($n = 9$) were 98.63±0.34, 99.61±0.58, 99.35±0.58 and 99.72±0.86 for tablets, ophthalmic ointment and cream, respectively. A proposal of the reaction pathway was presented.

Keywords: Acyclovir; 3-Methylbenzothiazolin-2-one hydrazone; Ferric chloride; Pharmaceutical preparations; Spectroscopy; Pharmaceutical analysis

1. Introduction

Acyclovir [2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6H-purina-6-one; acycloguanosine; 9-[2-hydroxyethoxy)methyl]guanine, is an antiviral agent which is highly active in-vitro against herpes simplex (HSV) type-I and II and varicella viruses, but its toxicity to mammalian cells is low. Acyclovir is phosphorylated to the active compound acyclovir triphosphate after entry into a herpes infected cell. Intracellular conversion of acyclovir by viral thymidine kinase to the triphosphate which acts as an inhibitor for the herpes specified DNA polymerase preventing further viral DNA-synthesis without affecting normal cellular processes [1].



Acyclovir

Acyclovir is the subject of monographs in both the British Pharmacopoeia (BP) [2] and the European Pharmacopoeia [3]. Both methods recommended non-aqueous titration of the raw material using perchloric acid as a titrant. For the tablets and creams, the BP [2] described a spectrophotometric method based on measuring the absorbance of the extracts at 255 nm.

Several methods have been reported for the analysis of acyclovir either in pure form or in pharmaceutical forms as well as in the biological fluids and tissues, viz. spectrophotometry [4–6], HPLC [7–9], fluorimetry [10], radioimmunoassay [11,12] and enzymatic immunoassay [13].

3-Methylbenzothiazolin-zone hydrazone (MBTH), has been frequently used in pharmaceutical analysis,

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0014-827X/02/$ - see front matter
PII: S0014-827X(02)01299-5

thus, it has been utilized as a color producing reagent for determination of acetaminophen and phenobarbital simultaneously [14], ritodrine hydrochloride and amoxicillin [15], metoclopramide hydrochloride [16] and pefloxacin [17].

2. Experimental

2.1. Apparatus

Unicam UV–Vis spectrophotometer, Helios α, Cambridge, UK, with 1-cm quartz cells was used. The slit width was 0.2 mm.

2.2. Materials and reagents

- Acyclovir pure sample was kindly provided by Seduce Company, Cairo, Egypt.
- Tablets containing acyclovir: Zovirax 200, labeled to contain 200 mg acyclovir/tablet (lot no. A001543) and Zovirax 400, labeled to contain 400 mg acyclovir/tablet (lot no. A023142).
- Zovirax ophthalmic ointment, labeled to contain 3% w/w acyclovir (lot no. A019925).
- Zovirax cream, labeled to contain 5% w/w acyclovir (lot no. B2269DK). All were obtained from commercial sources.
- 3-Methylbenzothiazolin-2-one hydrazone (MBTH) (Sigma, St. Louis, MO (400 mg/100 ml 0.1 M HCl). It is stable for at least 2 days when kept in refrigerator.
- Ferric chloride hexahydrate (Riedel-de Haen, Germany) 1% aqueous solution was prepared.
- Hydrochloric acid (BDH Laboratory Supplies, UK, GPR^{TM}) a 0.1 M aqueous solution was used.
- Standard solution of acyclovir was prepared by dissolving 25 mg in 50 ml of water and was further diluted as appropriate.

2.3. Procedures

2.3.1. Recommended procedure and calibration curve

MBTH (1 ml) was transferred into a series of 10-ml volumetric flasks. Aliquot volumes of acyclovir standard solution was added so that the final concentration was in the range of 20–200 μg ml^{-1}, then 1 ml of ferric chloride solution was added. This solution was mixed and allowed to stand for 20 min. The volume was adjusted to the mark with water. The absorbance was measured against a reagent blank (which contains all reagents except acyclovir) at 616 nm. The absorbance versus the final concentration was plotted to get the calibration curve, or to derive the regression equation.

2.3.2. Procedure for the tablets

Ten tablets were weighed and pulverized. A weighed portion of the powder equivalent to 25 mg of acyclovir was transferred into 50-ml standard flask and shaken using a sonicator for 15 min. The volume was adjusted to the mark with water, mixed and filtered and then proceeded as described under Section 2.3.1. The nominal content of the tablets was determined either from the calibration curve or using the regression equation.

2.3.3. Procedure for ointment

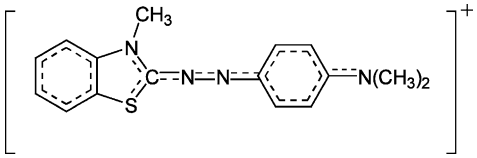
About 400 mg of acyclovir ointment was shaken with 50 ml of methanol in a 100-ml standard flask using a sonicator. The volume was completed with methanol and proceeded as described under Section 2.3.1.

2.3.4. Procedure for cream

Acyclovir cream (400 mg) was shaken for 30 min and centrifuged for 15 min with 50 ml of methanol. The volume was completed with methanol and proceeded as described under Section 2.3.1.

3. Results and discussion

3-Methylbenzothiazolin-2-one hydrazone (MBTH) was introduced as a reagent for colorimetric determination of pharmaceutical compounds in 1961. MBTH can react with carbonyl derivatives through its hydrazone grouping. On the other hand, it forms a strongly electrophilic diazonium salt when acted upon by an oxidizing agent. This diazonium salt can couple with various compounds. This method allows the determination of compounds containing primary aromatic amines, aralkylamines, aryldialkylamines, diarylamines, indoles, carbazoles and phenothiazine-type compounds. The following structure was attributed to the colored cation given by N,*N*-dimethylaniline [20].



Acyclovir was found to react with 3-methylbenzothiazolin-2-one hydrazone (MBTH) in the presence of HCl and the Fe(III) an oxidant, producing a deep-green color peaking at 616 nm (Fig. 1). The spectrophotometric properties of the colored product as well as the different experimental parameters affecting the color development and its stability were studied carefully and optimized. Such factors were changed individually while the others were kept constant. The factors include, concentration of the reagents (MBTH and $FeCl_3$), temperature, time of development of the color, effect of surfactants, sensitizers and acidity of the solution.

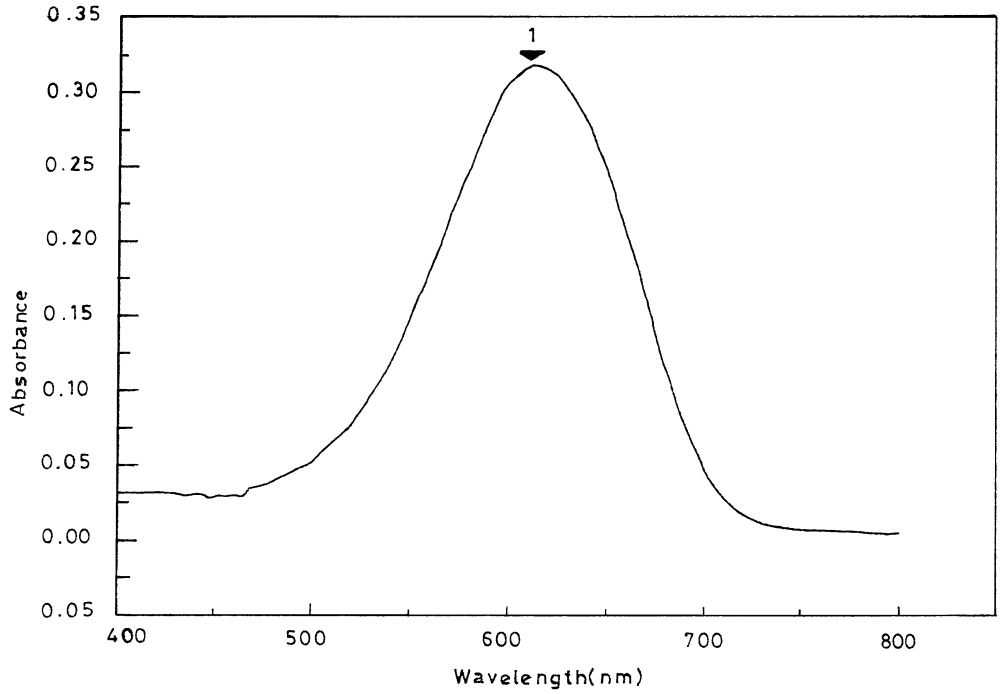


Fig. 1. Absorption spectrum of the reaction product of acyclovir (75 μg ml^{-1}) with MBTH–$FeCl_3$.

The influence of the concentrations of MBTH and $FeCl_3$ were studied using different concentrations. It was found that 1 ml of 0.4% solution of MBTH in 0.1 M HCl and 1 ml of 1% solution of $FeCl_3$ were optimum volumes for complete reaction (Fig. 2).

Effect of temperature on the development of the color was studied by heating the reaction solution in a thermostatically-controlled water bath. Different temperature settings were used with constant heating time. Increasing the temperature of the water bath, was found to produce decrease in color till it disappeared completely upon boiling, therefore, the reaction was conducted at room temperature. The time of the reaction is an essential part of the experiment. Different time intervals were attempted. It was found that 20 min after addition of MBTH and $FeCl_3$ was sufficient for reaching its highest absorbance. The absorbance of the color was found to be stable for at least 12 h.

Different sensitizers (quinine and fluorescein) at 5 μg ml^{-1} were tested by adding to the reaction mixture. It

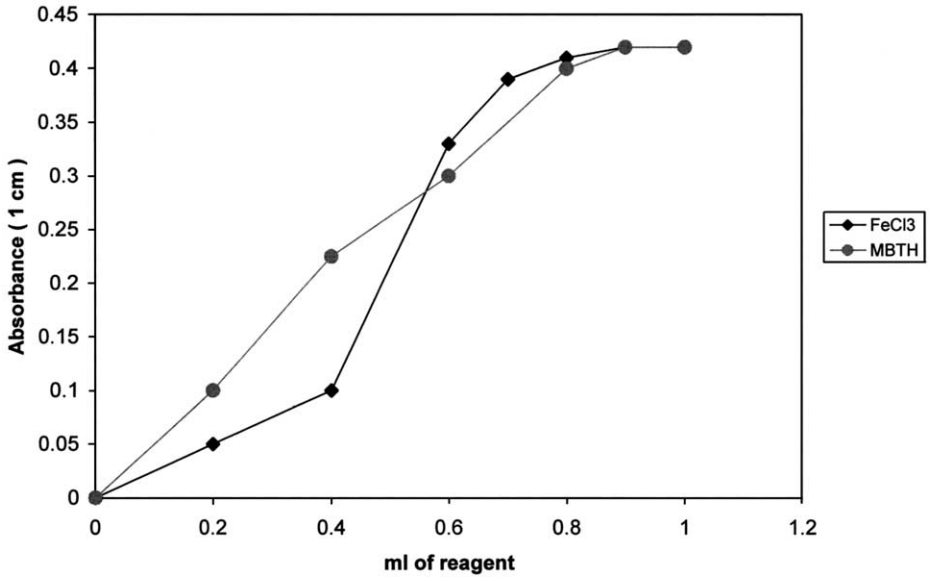


Fig. 2. Effect of the concentration of MBTH (0.4%) (at 0.1% $FeCl_3$ final concentration) and $FeCl_3$ (1%) (at 0.04% MBTH final concentration) on the reaction product with acyclovir (100 μg ml^{-1}).

Table 1
Effect of surfactants on the performance of the proposed method (using 80 μg ml^{-1} acyclovir)

Surfactant	Concentration (μg ml^{-1})	Absorbance
No surfactant	80	0.344
Cetrimide	2.0	0.343
Sodium lauryl sulphate	2.0	0.290
Gelatin	2.0	0.344
Cetrimide	4.0	0.338
Sodium lauryl sulphate	4.0	0.286
Gelatin	4.0	0.334
Cetrimide	8.0	0.340
Sodium lauryl sulphate	8.0	0.230
Gelatin	8.0	0.346

was found that color disappeared completely. In the same manner, the effect of surfactants (cetrimide, gelatin and sodium lauryl sulfate) at three different concentration levels (2, 4, 8 μg ml^{-1}) were tested by adding to the reactant mixture. It was found that cetrimide and gelatin had no effect on the absorbance reading while sodium lauryl sulphate had inhibitory effect as evident from the low absorbance readings (Table 1).

3.1. Analytical performance

The absorbance–concentration plot was found to be liner over the range 20–200 μg ml^{-1}. Linear regression analysis of the data ($n = 7$) gave the following equation

$$A = -0.0024 + 0.0043C \qquad R = 0.9998$$

where A is the absorbance in 1-cm cell, C is the concentration of the drug in μg ml^{-1}. The minimum detection limit is 1.06 μg ml^{-1} (4.71×10^{-6} M).

The apparent molar absorptivity was found to be 9.4×10^2 l mol^{-1} cm^{-1} and $A^{1\%}_{1\ cm}$ was ~ 42.

Statistical evaluation [18] of the regression line gave the following values: standard deviation of the residuals ($S_{y/x}$) is 5.495×10^{-3}; standard deviation of the intercept (S_a) is 1.037×10^{-3}; standard deviation of the slope (S_b) is 3.36×10^{-5}, while the percentage error is 0.25%. These small figures point out to the high precision of the proposed method.

The tolerance limit of amantadin (antiviral) which is frequently co-administered with acyclovir was calculated and was found to be 250 μg ml^{-1}.

The proposed method was applied to the determination of pure sample of acyclovir. The results obtained by the proposed method were compared with those given by the official method [2]. Statistical analysis [18] of the results obtained by both methods using the Student's t-test and variance ratio, F-test, reveals no significant difference in the performance of the two methods at the 95% confidence level regarding accuracy and precision, respectively (Table 2).

3.2. Pharmaceutical applications

The proposed method was applied successfully for the determination of acyclovir in some pharmaceutical preparations adopting the standard addition technique. In case of acyclovir tablets there was no problem during extraction of acyclovir with water, followed by filtration, and there was no interference from soluble excipients or additives. In case of acyclovir ointment and cream, there was no need for an extraction process because the bases are soluble in methanol giving clear solution without interference with the proposed method.

Table 3 assessed the validity of the method with regard to accuracy and precision and indicate that the proposed method can be successfully applied for the determination of acyclovir in pharmaceutical preparations without interference from additives or excipients.

Compared with the official methods [2,3] the proposed method is more specific, as it is based on measuring the absorbance of the reaction product at 616 nm. Thus no interference is encountered from excipients, degradation products or related substances whereas the official method which is based on measuring the absorbance at 255 nm is subjected to interference from any co-existing compounds that have the ability to absorb in the UV-region. So the proposed method can be considered as an alternative substitute to the official method.

Table 2
Application of the proposed method and official method to the determination of acyclovir in pure sample

Amount taken (μg ml^{-1})	Amount found (μg ml^{-1})	Recovery (%)	Official method
25	24.71	98.83	98.91
50	50.03	100.06	99.87
100	99.98	99.98	100.20
150	150.18	100.12	98.80
Mean ± SD		99.75 ± 0.53	99.47 ± 0.54
t		0.74 (2.45)	
F		1.038 (9.28)	

Each result is the average of three separate determinations. The figures in parenthesis are the tabulated values of t and F at $P = 0.05$.

Table 3
Determination of acyclovir in pharmaceutical preparations by the proposed method

Preparation	Standard addition		
	Amount taken (μg ml^{-1})	Authentic added (μg ml^{-1})	Recovery (%)
Zovirax 200® tablets (acyclovir 200 mg/tablet) [a]	50.0	40.0	98.30
	50.0	100.0	98.50
	50.0	150.0	99.10
Mean ± SD			98.63 ± 0.34
Zovirax 400® tablets (acyclovir 400 mg/tablet)	75.0	25	98.82
	75.0	75	100.19
	75.0	125	99.82
Mean ± SD			99.61 ± 0.58
Zovirax® ophthalmic ointment (acyclovir 3% w/w)	100.0	25	99.31
	100.0	50	100.08
	100.0	100	98.66
Mean ± SD			99.35 ± 0.58
Zovirax cream® (acyclovir 5% w/w) [b]	50	50	99.00
	50	75	100.67
	50	120	99.50
Mean ± SD			99.72 ± 0.86

Each result is the average of three separate determinations.
[a] Product of the Wellcome Foundation Ltd., London.
[b] Product of Glaxo Wellcome Operations, Greenford, Middlesex, UK.

3.3. Mechanism of the reaction

The stoichiometry of the reaction was studied adopting the limiting logarithmic method [19]. The absorbance of the reaction product was measured in the presence of excess of both MBTH and acyclovir. A plot of log absorbance versus log [MBTH] and [acyclovir] gave straight lines, the values of the slopes are 1.003 and 0.991, respectively (Fig. 3). Hence, it is concluded that, the molar reactivity of the reaction is 1.003/0.991, i.e. the reaction proceeds in the ratio 1:1. Since the reaction involved coupling with $-NH_2$ group, it needs a slight acidic medium [20].

4. Conclusions

Acyclovir has been determined by a specific and simple method. The drug can be determined in the presence of its degradation products, related substances and irrelevant coexisting substances and excipients. It allows determination of down to 20 μg ml^{-1} with good

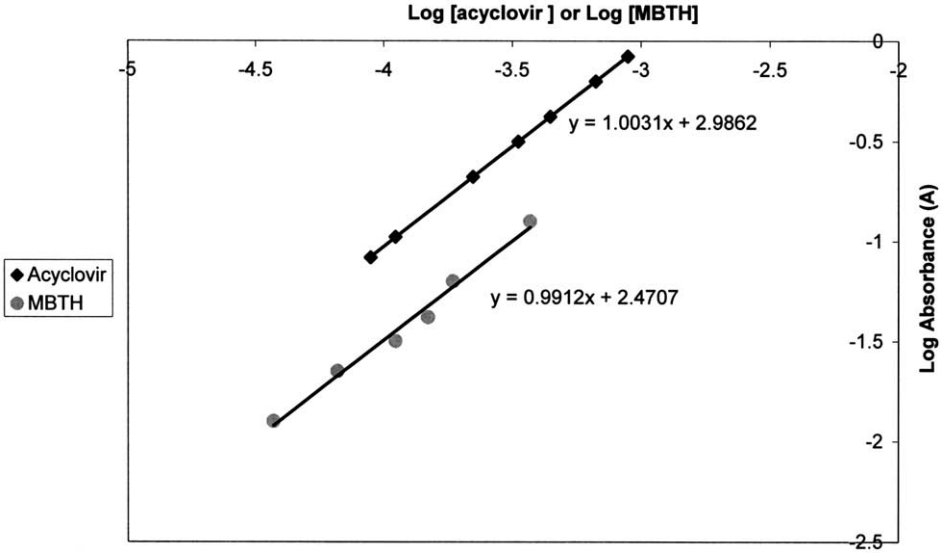


Fig. 3. The limiting logarithmic method for acyclovir with MBTH/$FeCl_3$.

accuracy and precision. The lower limit of detection (1.06×10^{-6} M) is comparable to that reported by other methods. It can be readily adopted to routine control analysis of the drug in its formulations.

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