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Development of Batch and CFIA/Merging Zones Procedures for Estimation of Ganciclovir Drug in Pure Material and Pharmaceutical Preparations using Potassium Permanganate as an Oxidizing Agent

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Abstract: ***A new batch and FIA- merging zones spectrophotometric procedure were developed for the assay of ganciclovir (GCV) in a pure drug and in pharmaceutics preparations. These procedures are characterized by speed , sensitive accuracy and simplicity. The procedure was based on the oxidation of ganciclovir using potassium permanganate as an oxidimetric reagent in alkaline medium to composed a green colored complex was measured at λmax of 612 nm. The optimized FIA system was able to determine of GCV with throughput 58 sample/h, with 1.2 mL.min^{-1}flow rate employing distilled water as a carrier of chemicals. The calibration curves of absorbance against concentration demonstrate that beer's law is submitted within the concentration scope of 3-90 & 2-250 mg. L^{-1} of ganciclovir with detection limits 0.18 and 0.013 mg. L^{-1} of ganciclovir for batch and CFIA procedures, respectively. The suggested method was carried out successfully for estimation of ganciclovir in dosage forms. The recently developed procedure statistical assessed with standard procedure and there were no considerable contrasts between either procedures .The developed procedure can be adopted as an alternate analytical procedure for estimation of ganciclovir in pharmaceutics samples.***

Keywords: Ganciclovir , CFIA / Merging Zones, $KMnO_4$, Spectrophotometric determination, Redox reaction

1. Introduction

Ganciclovir (GCV) is chemically2-amino-1,9-[2-hydroxy-1-(hydroxymethyl) ethoxymethyl]-6H-Purine-6H-one, as shown in figure (1). It is an a cyclic guanosine analogue that needs triphosphorylation for stimulation previous to preventing the viral DNA polymerase and is employed for the curing of cytomegalovirus (CMV) infection in helpers sicks [1-4]. Ganciclovir exhibits antiviral activity opposition impetigo simple virus (HSV) and cytomegalovirus (CMV) at comparatively low inhibitory concentrations. GCV is a strong inhibitor of all herpes viruses containing ; Epstein-Barr virus and cytomegalovirus [5]

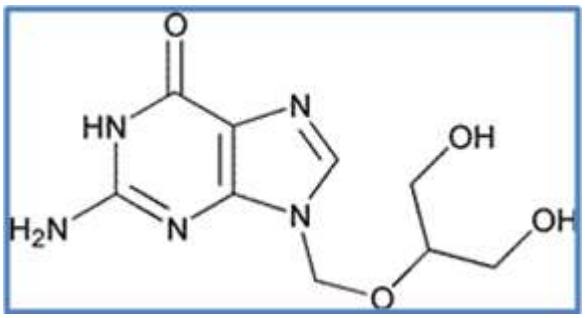


Figure 1: The chemical composition of ganciclovir

GCV is a white crystalline powder with a molecular formula of $C_9H_{13}N_5O_4$ and a molecular weight 255.23 g/ mole [1]. Literature survey reveals that few methods like capillary electrophoresis method which was used for GCV estimation in human plasma[6] or pharmaceutical preparations which include HPLC [7-9] square wave and differential voltammetry[10] enzyme-linked immunosorbent assay [11,12] radioimmunoassay[13] . These techniques require sophisticated instruments , involve several manipulation steps , a high expensive organic reagents and derivatization chemical reactions. Finally, spectrophotometric methods continuously to be the best preferred and developed techniques for routine analytical work for estimation of GCV in pure drug and its dosage forms because their reasonable sensitivity , simplicity and a long with significant economical advantages. GCV was determined spectrophotometrically analysis just as the reaction of GCV with p-dimethylaminocinnamaldehyde[14] and with several π and σ acceptors , GCV reacts as n-electron donor through a charge transfer reaction[15] first order derivative spectroscopy[16] and in acidic mediums , GCV can be oxidized using different inorganic oxidants[17] and in alkaline medium , the reduction of potassium permanganate by GCV[18] also GCV determination using CL-FIA[19-21] . $KMnO_4$ has been used in pharmaceutical analysis as oxidizing agent for colorimetric determination of Amlodipine besylate[22], Furosemide[23], Pantaprazole[24], Ciprofloxacin and lomefloxacine[25], Olnazapine[26] , Labetalol[27], Tetracycline[28] ,Enalapril maleate[29] , Pipazethate and dextromethorphan[30] , Piroxicam and Tenoxicam[31] , Metronidazole benzoate[32] and Hyoscine butylbromide[33]. The present work deals with the developed of CFIA/Merging Zones spectrophotometric determination of ganciclovir using potassium permanganate as oxidizing agent in presence of NaOH as basic medium through the redox reaction of GCV. The proposed method in this article paper was characterized by ; can reduce the time needed for analysis through use of mechanical processes instead of manual such as separation and mixing steps , it was capable

of sampling rate analysis at the rate of 100 sample/h with high repeatability and accuracy in the readings.

2. Experimental

Apparatus and manifold
A Shimadzu UV-Visible 1800 (Tokyo- Japan) a digital double beam recording spectrophotometer with (1cm quartz) cell. The flow cell (quartz, 1cm) with 100 µL internal volume is inside the detection unit and (1cm) an optical path length using for the absorbance measurements.

All of spectral absorbance quantifications were applied on the optima VIS-300 digital single beam that record spectrophotometer for CFIA procedure as peak height through kompensograph C1032 siemens or absorbance with digital multimeter (DT9205A, China). A one line system was used for FIA-merging zones spectrophotometric for estimation of ganciclovir with flow cell (quartz , 1cm , 100 µL internal volume) which it fixed inside the detection unit . A power supply (Yaxun, 150AD, China) with Peristaltic pump (Master flex C/L, USA) that performed to pump of the carrier of distilled water where all of the chemicals , sample and reagent are loaded in an injection valve (homemade, six-three way) contain three loops (Teflon) (merging zones version)) [(34)] that moves at 90^{o} .

The injection valve was used to supply suitable volumes of standard solutions that injected and sample. The tubes were made of flexible vinyl with 0.22 mm (I.D) was employed for the peristaltic pump and 1mm for connect the parts of manifold system (made of Teflon). The whole of parts of the CFIA/ Merging Zones system was shown as in Figure (2) . A carrier stream was distilled water (D.W) that was combined with injected sample (Ganciclovir , L_1) mixed with sodium hydroxide (L_2) as basic medium and potassium permanganate in (L_3) as oxidizing agent of drug . All of them were merged in reaction coil with length (100cm) , injection sample (51µL), flow rate of carrier ($1.2ml.min^{-1}$) . The absorbance was quantified at λmax = 612 nm. As pk.hgt. expressed in mV. (n=3).

Chemicals and reagents
Chemical and reagents were used of analytical class and all the solutions freshly preparing always used.

Ganciclovir stock solution (M.wt=255.23 g/mole) was provided from European directorate for the quality of medicines and health care (500 µg.ml^{-1} = 1.95x10^{-3}M).
A 0.05 g amount of a pure GCV dissolving in D.W , then consummated to 100 ml of standard flask with distilled water, more diluted solutions preparing by adequate dilution of the stock standard solution with D.W .

Potassium permanganate ($KMnO_4$) (M.wt=158.34 g/mole , Merck , Darmstadt , Germany) ($4.5x10^{-3}$M) ;
A 0.07125 gm of potassium permanganate dissolving in D.W and dilute to 100 ml in a calibrated flask , then standarized against to sodium oxalate ($Na_2C_2O_4$) in acidic medium[(35)].

Sodium hydroxide (NaOH) (M.wt = 40 g/mole , 1M , Merck)
A 4 gm of NaOH dissolving in D.W and dilute to 100 ml of D.W.

Procedure for the assay of Ganciclovir in commercial tablets (500 µg.ml^{-1}) :
Pharmaceutical formulations were gained from trading sources available either injection (vial) , capsule or tablets by selecting 10 capsules from three kinds companies were assayed by the suggested method , including:

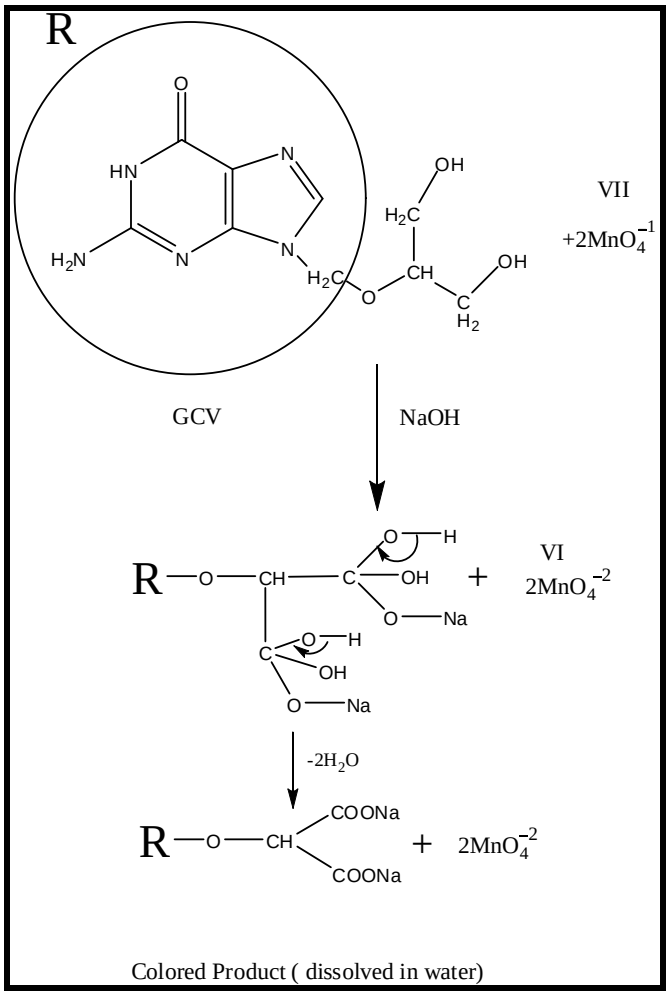
1-Lovir capsules 250 mg, Oubari pharma. Aleppo (Syria). 2-Lovir tablets 400 mg, Pack-In house pharmacy/VU. 3-Cymevene (IV) 500 mg vials, Roche , Australlia.

An accurately weighed quantity of the mixed content of 10 capsules , the tablets were weighted also an accurately , exterminated and milled employing motor up to become good powder .A 0.05 gm of the each sample was weighting that be equal to 500 mg. L^{-1} standard solution of an activated component for each dosage forms. This amount of GCV was dissolving in D.W and filtrated to removal the insoluble residue that affect on the analytical signal. The filtrate transferring in 100 ml standard flask and conclude to the marked with D.W,; further solutions were diluted and solutions preparing to allotment the concentration inside of the linearity of the calibration graph.

A vial of dosage forms (injection) ; the content of two vials were mixed and amount 0.05 g was weighting that be equal to 500 mg. L^{-1} standard solution of GCV and dissolved in D.W and exuded , the volume was concluded to 100 ml standard flask with D.W , from this standard solution 20 ml by the pipette into 100 ml standard flask and the volume be completed to the marked with D.W to prepare the concentration of 100 mg.L^{-1}. The solution was continued to do as represented under method for calibration.

3. The Proposed Mechanism

The mechanism of reaction is an oxidation / reduction reaction[(18)] of GCV with potassium permanganate in sodium hydroxide as an alkaline medium to produce a green colored product was measured at λmax of 612 nm , may be happens as in a scheme (1) :



Scheme 1: The proposed mechanism of the reaction of GCV with $KMnO_4$

The stoichiometry of the reaction between GCV with $KMnO_4$ as an oxidizing agent was studied using mole ratio and continuous variation[36] methods . The results obtained show that the colored product was formed in the proportion of 2 : 1 ($KMnO_4$:GCV) as it is apparent in Figure (2) and (3) . By mole ratio , the conditional stability constant (K) was calculate and found to $4.25x10^8$, indicating good agreement with high stability of the [GCV-OH^--MnO^-_4] complex.

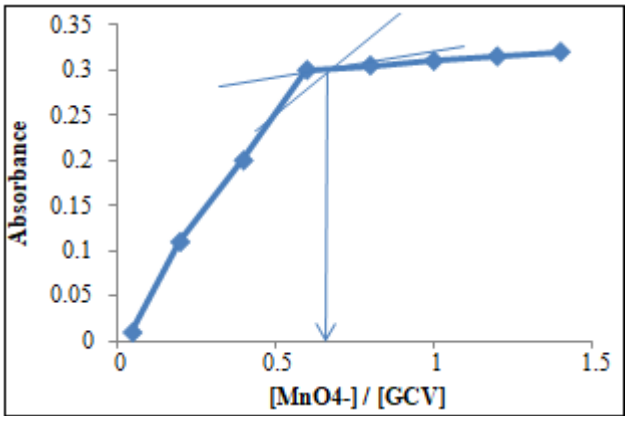


Figure 2: Mole ratio plot for reaction of GCV with MnO_4^{-1}

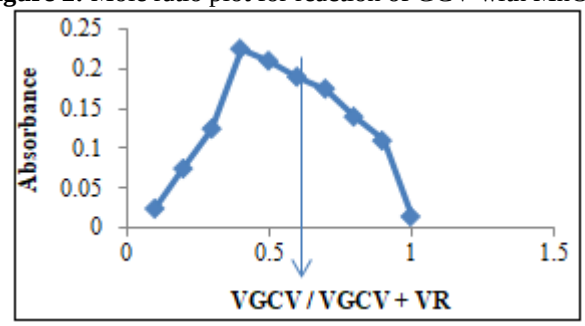


Figure 3: Continuous variation plot of the between GCV and $KMnO_4$ employing batch method

4. Procedures and Construction of Calibration Graph

Batch method

A ganciclovir solutions in the range (3-90) mg / L were prepared from the stock solution 500 $mg.L^{-1}$ into 10ml volumetric flasks, then added 1.5ml of NaOH (1M) followed by 1.2 ml of $5x10^{-3}$M $KMnO_4$ and mix well , then complete the solution to the marked with distilled water and the reaction mixture to stand for 10 min . After this time, the absorption of the colored complex was found under λmax 612 nm opposition the reagent blank was prepared simultaneously.

FIA / Merging Zones method

A set of 10 ml standard flasks, different volumes of GCV standard solution covering in concentration range (2-250) mg / L were prepared from the stock solution 500 mg /L. The injection volumes of 51µL in L_1 , 49.06µL in L_2 , 43.18 µL in L_3 were loaded of ganciclovir in L_1 , Sodium hydroxide in L_2 and Potassium permanganate in L_3 . One channel of manifold FIA system using (D.W.) as carrier , the analyte and other chemicals of loops be injected to system with flow rate 1.2 ml/min. The resulting absorption of the colored complex (green) be found under λmax 612 nm opposition the reagent blank (518nm) which was carried out under the same condition without drug.

5. Result and Discussion

Batch spectrophotometric determination

Experiments were oriented for ideality of the experimental parameters for create the ideal parameters for quantitative and rapid composition of the colored complex with highest sensitivity and stability ideality of the experimental conditions .The reaction of ganciclovir (25mg/L) with potassium permanganate ($3x10^{-4}$M) as an oxidizing agent for the drug in alkaline medium (0.15M NaOH) to compose a green colored was found at λmax 612 nm opposition reagent blank as shown as in figure (4)

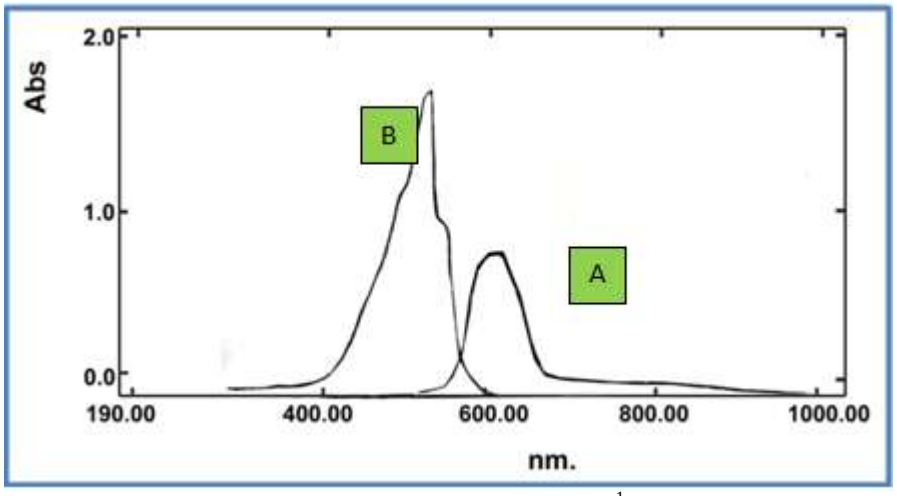


Figure 4: A- Absorption spectra of colored product formed [GCV-OH^--MnO_4^{-1}] opposition reagent blank , B - Reagent blank opposition distilled water. (25 mg/L of GCV)

Optimization of conditions

The volume of $KMnO_4$ has a great effect on the formation of GCV-$KMnO_4$ complex (figure 5) shows that 1.2 ml ($6x10^{-4}$M) of $KMnO_4$ was sufficient to give a maximum absorbance, using 25mg / L GCV were studied.

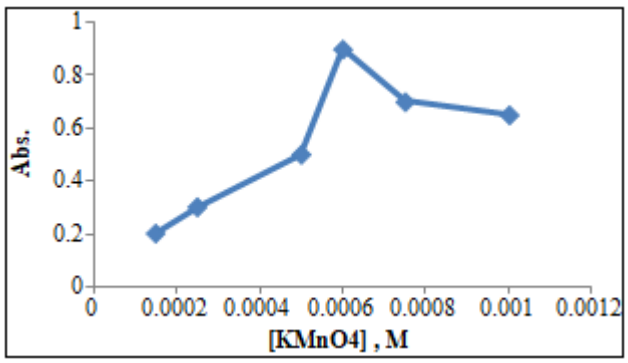


Figure 5: Effect of $KMnO_4$ concentration on the response

To generate the nucleophilic from ganciclovir and also to activate the nucleophilic substitution reactions, alkaline medium is more necessary for the reaction with potassium permanganate . Different inorganic bases were examined, in order to obtain high sensitivity and selectivity for the estimation of ganciclovir ; such as NH_4OH , KOH , NaOH , Na_2HPO_4 and Na_2CO_3. It was found that using of sodium hydroxide gave a highest intensity (table (1)) where with other bases either precipitation of white colloid accurs upon diluting the reaction with an oxidizing agent , non reproducible results , or high blank readings and weak sensitivity were observed.

Table 1: Effect of base on the reaction of GCV with $KMnO_4$

Type of alkaline Medium (1M)	Absorbance
NaOH	**0.648**
KOH	0.487
Na_2HPO_4	0.235
Na_2CO_3	0.234
NH_4OH	0.093

A study for optimization of sodium hydroxide concentration reveals that the optimum concentration was 0.12M (1.2 ml). Therefore , this amount was used in this study as shown in figure (6).

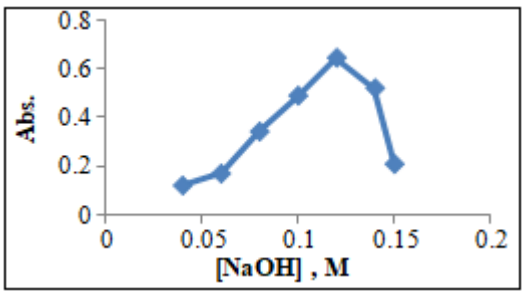


Figure 6: Influence of NaOH concentration on the reaction of GCV with $KMnO_4$

The stability of the absorbance with time was studied from 5-60 min. This study showed that the colored dye was developed at 10 min . After the addition $KMnO_4$ to GCV in alkaline medium , the absorbance remained stable for at least 6hr.

All investigated terms were ideal at the temp.($25C^0$) under that the best color intensity was gained as shown in Figure (7)

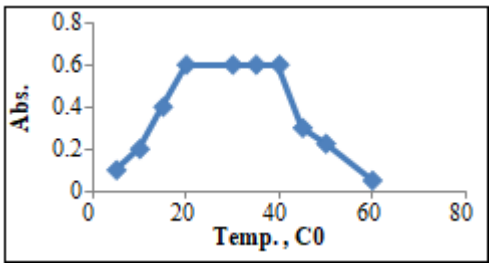


Figure 7: Effect of temperature on the absorbance of [GCV-OH^-- MnO^-_4] complex

Ganciclovir-NaOH-$KMnO_4$ is the ideal sequence of addition compared with other sequences under the same experimental conditions (table 2)

Table 2: Effect of sequence additions

Reaction components	Abs.
GCV- $KMnO_4$- OH^-	0.257
GCV- OH^-- $KMnO_4$	**0.643**
$KMnO_4$- OH^-GCV	0.348

The influence of different types of surfactants [37] including Cetavlon , Triton x-100 (1%) , Sodium dodecyl sulphate (0.1%) and Cetyltrimethylammonium bromide (CPC) were tested. Non of them make better the absorption intensity; therefore they were eliminated from this study.

In pharmaceutical analysis procedures ; it is important to examine the selectivity of the methods towards additives and excipients added to the dosage forms of ganciclovir. Table (3) shows that the commonly encountered excipients don't interfere with the developed method demonstrating a high selectivity for determining of GCV in its pharmaceutical formulations.

Table 3: Influence of additives for estimation of GCV (25mg/L)

Additives	*Rec.% of additives added	
	500	2000
Croscarmellose Sodium	102.46	103.34
Microcrystalline Cellulose	100.86	100.76
Starch	101.25	103.28
Magnesium Stearate	100.32	101.64

***Average of three determination**

Quantification of classical method

At the empirical terms , standard calibration curve for GCV be created by plotting the absorption against the concentration as shown in figure (8) . Beer's law was submitted to concentration (3-90) mg / L for the determination of ganciclovir , each measurement was repeated three times as shown in figure (8).

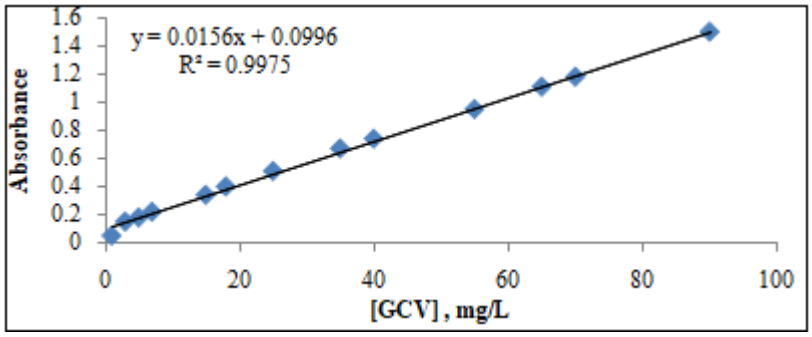


Figure 8: Calibration curve of GCV using classical procedure

Rec% (the recovery) and the RSD% (relative standard deviation) were determined at three various concentrations of ganciclovir to estimate the accuracy and precision of the suggested procedure , these values shown in the table (4) indicate a high accuracy (av. rec% =100.15) while the RSD% is < 1.0% .

Table 4: Accuracy and precision of the classical procedure

Taken , μ (mg/L)	Found , $\bar{x}$ (mg/L)	Error	$E_{rel.}$%	*Rec.%	*RSD%
10	10.06	0.06	0.6	100.6	0.71
20	19.95	-0.05	-0.25	99.75	0.04
40	40.04	0.04	0.1	100.1	0.00

***Average of five determinations**

The manifold of flow injection-merging zones system

After installing the system and linked portions , the optimal design of flow injection system were studied. The developed manifold of CFIA as shown in figure (9) which is composed of one line manifold was used for the flow injection analysis/merging zones spectrophotometric estimation of ganciclovir. A carrier stream (D.W.) at flow rate 1.2 ml / min that was joined with injected sample (GCV in L_1) ; mixed with alkaline medium (NaOH in L_2) and with an oxidizing agent ($KMnO_4$ in L_3) . All of the chemicals are loaded in an injection valve (homemade, six-three way) contain three loops (Teflon) (merging zones version) that moves at 90^o .

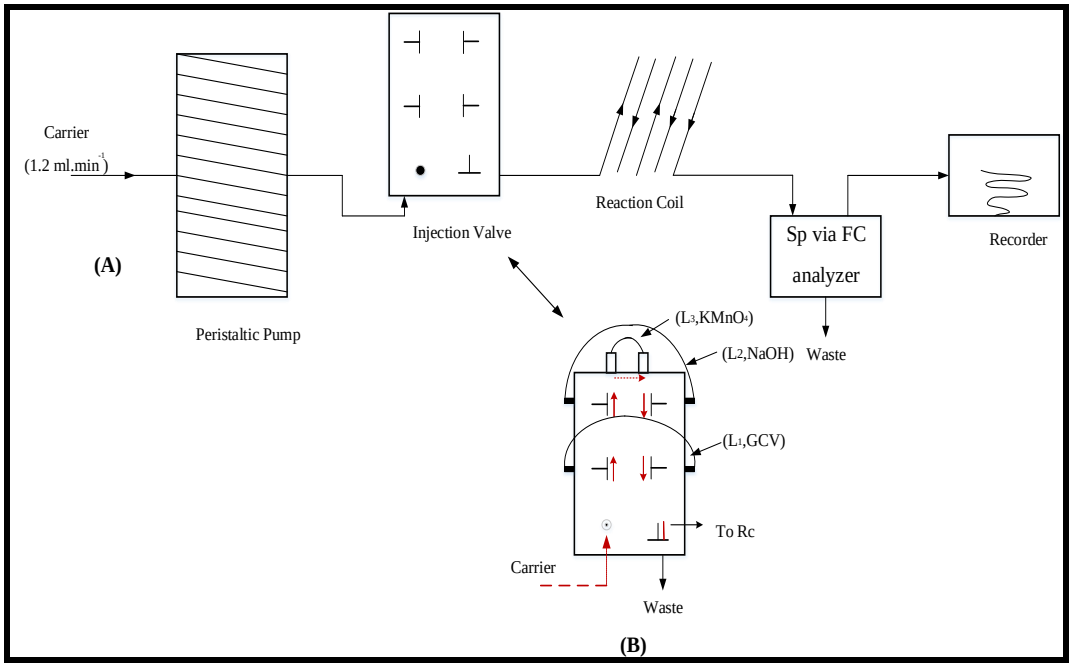


Figure 9: The diagram of CFIA/Merging zones technique using for spectrophotometric determination of GCV drug
A / CFIA system B / Injection valve (details with chemicals)

Optimization of experiment parameters

As shown in figure (9) , the flow injection manifold was employed for the optimization of chemical and physical parameters to obtain variables for the system for estimation of GCV drug in pure material and it's their pharmaceutical preparations. All the parameters were investigated by making all factors constant , change one each at time (single varied optimization)

The impact of potassium permanganate reagent was observed by taking different concentration ($2.5x10^{-4}$-$1.5x10^{-2}$ M) of reagent that was added to an aliquot of solution including 25 mg/L of ganciclovir (51µL sample volume injected) . All measurements were repeated for three successive times. Figure (10) shows that $8x10^{-4}$ M of $KMnO_4$ is the optimum concentration , at high concentration of an oxidizing agent, the absorbance as peak height will be decreases ; therefore, it was used in subsequent work.

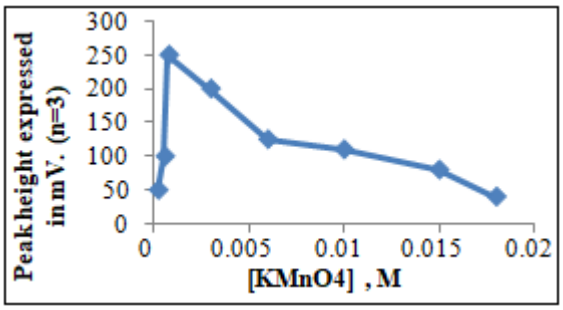


Figure 10: Effect of Potassium Permanganate Concentration

Various concentration ($2x10^{-2}$-$1.5x10^{-1}$M) of NaOH was examined on the analytical signal of the reaction product of 25 mg/L GCV drug (51µL sample volume) with $8x10^{-4}$M $KMnO_4$ (43.2µL) were used and the data obtained as shown in figure (11) , therefore ; the optimum NaOH Conc. of 0.1 M was selected in this study (49.1 µL in L_2)

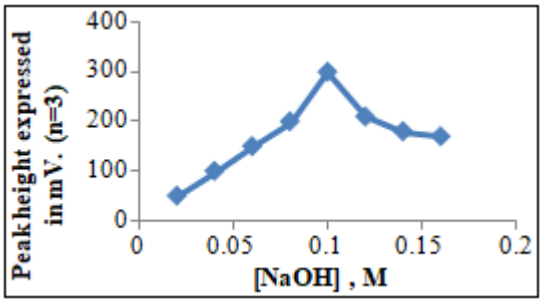


Figure 11: Influence of NaOH solution

The impact of the pH of GCV was studied and the results obtained to be 10.8 . Following the addition of $1x10^{-3}$ M $KMnO_4$, the value of pH be reduced to 10 for this reason , various buffers of pH=10 such as carbonate , phosphate and borate that preparing . It was found that these buffers suppression of the absorbance

Manifold variables

The impact of variables like volume of sample injected , volume of reagent , purge time , flow rate and length of reaction coil on the analytical response was investigated. The peak height based on the stay time of the sample in the system which was conducted with lengths for reaction coil and flow rate. The physical variables were studied under the optimum concentration of the reactants ; (25mg/L of ganciclovir, $8x10^{-4}$ M $KMnO_4$, 0.1M NaOH).

The effect of flow rate was studied under the optimum chemical parameters ; The results obtained (figure (12)) shows that the optimum flow rate of pump of the sample with least dispersion under 1.2 $ml.min^{-1}$ flow rate of carrier (D.W). At lower flow rate, the dispersion will be the highest level whereas at greater flow rate ; the reaction may be not complete , the absorbance as peak height were decreased.

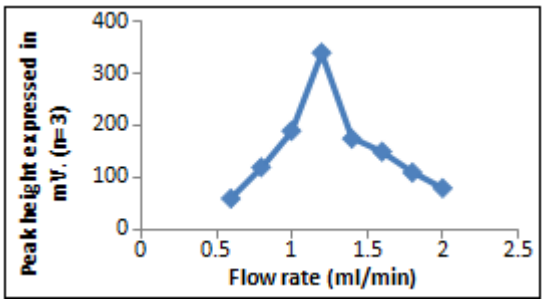
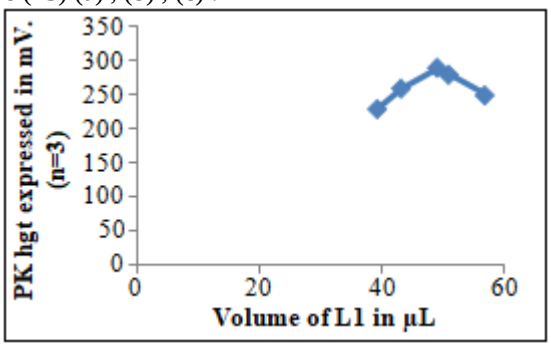
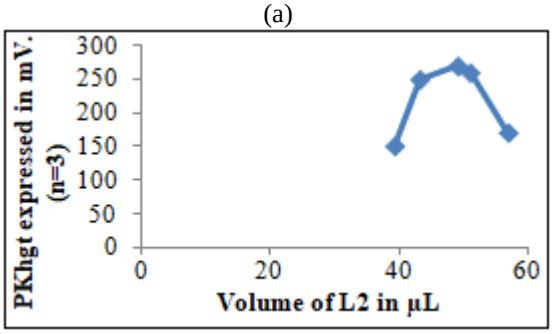


Figure 12: The variation of response as peak height with flow rate

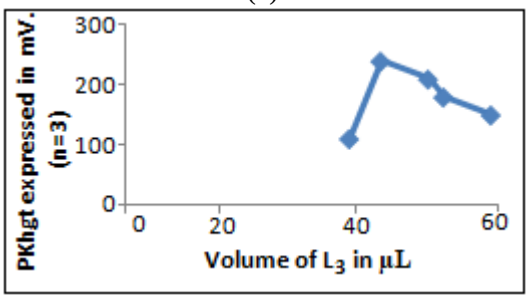
The injected volume of sample and reagents were investigated by using different volumes (56.9, 51.0, 49.1, 43.2 and 39.3) µL employing open valve model .The values obtained shows that injected volumes of 51.0 , 49.1 and 43.2 µL for sample (25 mg/L GCV) in **L1** , 0.1M NaOH in **L2** and $8x10^{-4}$ M $KMnO_4$ in **L3** , respectively were optimum volumes that presented the maximum signal as shown in Figure (13) (a) , (b) , (c) .



(a)



(b)



(c)

Figure 13: Effect of sample & reagents volumes on analytical response

The effect of different lengths of R.C (100 , 150 , 200 , 250) with internal diameter of 2mm that was placed after injection valve directly in the developed of FIA system ((figure 9)) . The optimum concentration of GCV was used for oxidation/ reduction of ganciclovir (25mg/L) in 0.1M NaOH with $8x10^{-4}$ M $KMnO_4$. It was observed that the response was decreased with the reaction coil length up to 100 cm due to the dispersion phenomena as shown in figure (14) . Therefore, a 100 cm gave the highest peak height and was used in all subsequent experiments.

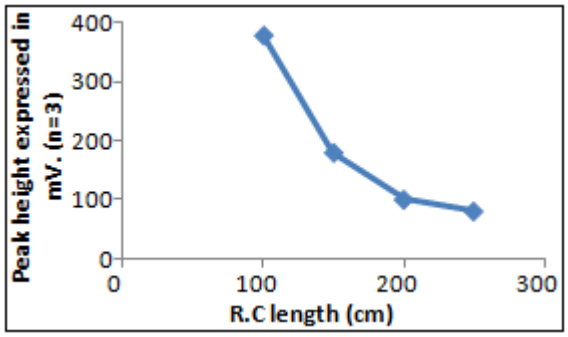


Figure 14: Influence of reaction coil length

The sample of each segment to be injected via a carrier stream through a homemade injection valve was calculated. Purge time is defined as the period time between the sample that was injected and emergence of the end of the response, using the optimum chemical and physical parameters were studied previously. A 5, 10, 15, 20, 25, 30 sec and open valve were used for this study. Open valve was selected (figure (15)) as optimum injection time with a highest response intensity to complete transportation of sample loop to measuring unit. The reaction time of sample of GCV drug was 62 sec, so the sample throughput was 58 sample / h .

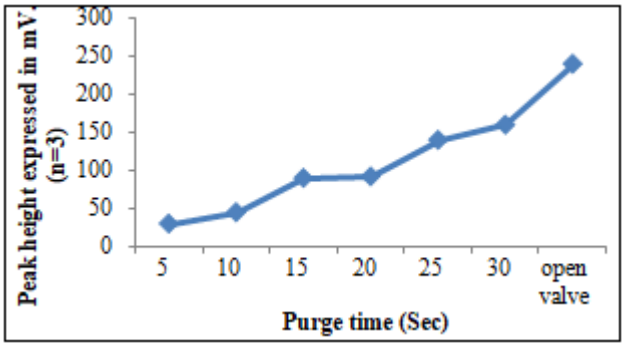


Figure 15: Influence of purge time on reaction of GCV with $KMnO_4$

The impact of temperature on the colored complex was observed under 5, 25 , 35 , 40 and 45 C^0 . The values indicated that the oxidation of ganciclovir was accured at 35 C^0 (room Temp.) with high stability of colored complex at least 60 min . (Ksta. $=1.4x10^9$ $L^2.mole^{-2}$), as shown in figure (16).

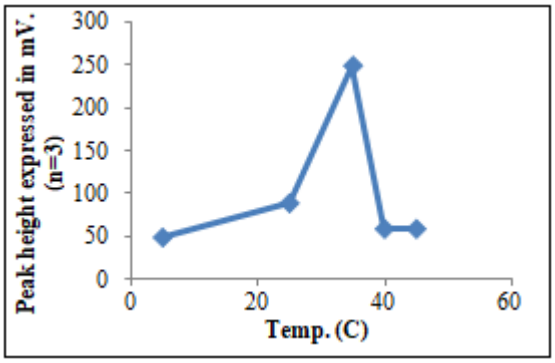


Figure 16: Effect of Temp. on response of colored complex

Analytical parameters

At the ideal terms depicted previously , calibration curve for Ganciclovir (figure (17)) was created by layout av. peak height in mV. as a function of the sample concentration. The linear calibration curve for the singular estimation in the scope of 2-250 mg. L^{-1} for GCV.

Analytical features such as linear scope , relative standard deviation , correlation coefficient , detection limits confidence limits of standard deviation of slope and intercept of each method were analyzed[38]

Statistical assessment of regression line presented the result of standard deviation for residuals (Sy/x) ; slope (S_b) and intercept (S_a) under 95% confidence for (n-2)[39] freedom degree were clarified in the table (5) . The small points were showed to elevated reproducibility and repeatability of the proposed CFIA contrasted with the classical method. CFIA / merging zone is easier than the batch procedure causes of it's wider linear range of calibration curve , speed (sampling through put of 58 sample/h) and good recovery with less error were obtained.

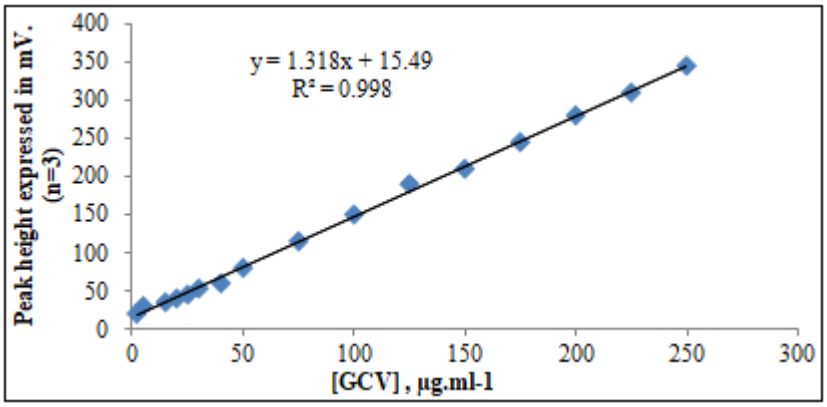


Figure 17: Calibration curve for determination of Ganciclovir using the proposed method

Table 5: Quantitative analytical and regression parameters of the suggested procedure for estimation of GCV

Parameters	Batch method	FIA method
Regression equation , y = b x+ a	y=0.0156x+0.0996	y=1.3182x+15.492
Correlation Coefficient / r^2	0.9975	0.9985
Linearity (r^2%)	99.75	99.85
Relative standard deviation(RSD%)	0.0136 (20 mg/L)	0.0635 (80 mg/L)
Slope (b) ; (L/mg)	0.0156	1.3182
Intercept; (a) ; (a = y– b x)	0.0996	15.492
Confidence limits of Intercept, a=a ±tS_a	0.0996 ± 0.0146	15.492 ± 4.2269
Confidence limits of Slope, b = b ±t S_b	0.0156 ±0.0041	1.3182 ± 0.0241
Standard deviation of the slope (S_b)	$9.6x10^{-4}$	$5.6x10^{-3}$
Standard deviation of the intercept (S_a)	$3.4x10^{-3}$	0.983
Limit Of Detection (LOD)	0.18	0.013
Limit Of Quantification (LOQ)	1.82	0.134
Average of recovery	99.3	100.2
Sampling (through put) /h	6	58

Assay of pharmaceutics samples

The developed procedure were performed for the quantitative estimation of ganciclovir in pharmaceutics preparations . Three kinds (various origins of these formulations) including ganciclovir were assayed, the results obtained were summarized in table (6) . They presented a good precision and accuracy with no interference from the excipients compared with those gained by standard procedure[40]. The results gained by the suggested procedures by performing the t-test & F-test under 95% confidence levels . The computed values for F-test were 0.234 & 1.65, t-test values were 0.66 and 1.39 for the batch &CFIA procedures respectively , didn't beyond the critical values of F-test=19.0 and t-test=2.77 (n_1+n_2-2) =4 . These confirmations that there are no important contrasts between the developed procedure and the standard procedure with regard to accuracy and precision for estimation of ganciclovir in pharmaceutics preparations, as shown in table (6) .

Table 6: Application of the suggested procedures and official procedure for the estimation of GCV in pharmaceutics formulations

	Suggested methods						
	CFIA method			Batch method			
Pharmaceutical formulation	Present Conc. (mg/L)	*Rec%	*RSD%	Present Conc. (mg/L)	*Rec%	*RSD%	Official Method (Rec%)
Lovir capsule (250mg) Oubari pharma Aleppo (Syria)	10 30 60	99.95 100.00 99.80	0.06 0.00 1.00	5 20 50	100.00 100.20 99.90	0.00 0.012 0.023	99.2
Lovir tablet (400mg) Pack-In house pharmacy/VU.	10 30 60	100.30 99.98 101.00	1.30 0.76 0.014	5 20 50	98.00 96.50 101.00	0.07 1.20 0.14	98.96

Cymevene (IV) vial (500 mg) Roche Australlia	10 30 60	99.80 98.90 100.00	1.25 0.20 0.00	5 20 50	99.40 100.00 100.30	0.24 0.00 1.60	99.4

6. Conclusion

The suggested homemade CFIA /merging zones analytical procedure are characteristics , rapid , sensitive and inexpensive for the spectrophotometric estimation of GCV in pure form and pharmaceutics formulations employing potassium permanganate as a suitable oxidizing agent without needs for previous separation steps, solvent extraction , temperature or pretreatment of sample. The main advantages of the proposed methods are its a large dynamic range , adequate sensitivity and it's suitable for employ in daily assay in pharmaceutics quality control laboratories due to their simplicity and less reagent consumption when contrasted with batch methods [18,37] and lowest of limit detection compared with the reference method[40] as well as the procedures have good linearity, high analytical frequency with sampling 58 sample/h .

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