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New Mode for the Determination of Ketotifen Fumarate Using Low Pressure Mercury Lamp via Continuous Flow Injection Analysis

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ABSTRACT
The method was based on the quenched of continuous fluorescence of fluorescence dye (N-(4-Aryl diazenyl-p-phenol-1,8-naphthal imide) that were used for determination of ketotifen fumarate. A comprehensive detailed study was carried out by ISNAG-fluorimeter CFIA that capable of measuring fluorescence emitted from fluorescent molecules that be excited by low mercury lamps. The linearity range, r,r^2,R^2% and limit of detection were : 0.01-10mmol.L^{-1} , 0.9837 ,0.9676 , 96.76 and 0.425 µg/125µL.Pure and pharmaceutical formulation of mentioned drug was analyzed successfully in this work . A comparison was made between the official quoted value and the practically found values for the three kinds of drug by individual t-test. Individual t-test reveals that there was no significant difference between both means (quoted value with calculated t-value) from developed method.

Keywords: Ketotifen fumarate, ISNAG – fluorimeter, Low pressure mercury lamp

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INTRODUCTION
Ketotifen fumarate (KTF) (4-(1-methyl-4-piperidylidene)-4H-benzo [4,5] cyclohepta [1,2-b] thiophene-10 (9H)- one hydrogen fumarate)(fig.1) is a second generation H1-antihistamine that is used commonly for the treatment of allergic conjunctivitis or the itchy red eyes caused by allergies and mast cell stabilizer which demonstrates greater permeability across the blood brain barrier than newer agents in the therapeutic class. It is most commonly solid as a salt fumaric acid and is available in two forms[1-4]. Several methods for determination of KTF have been reported such as spectrometry[5], selective electrodes[6], chemiluminescence[7], capillary electrophoresis[8], turbidity[9] and fluorometry[10].Fluorescence instrument which depend on fluorescence process with presence of fluorophore organic molecules which contain several combined aromatic group, or plane or cyclic molecules (several π bonds) that play important role by re-emit light upon light excitation like anthracence, fluorescein , rhodamine B and quinine was used in many analytical procedures involve quenching of fluorescence (decrease of fluorescence by interaction of the excited state of the fluorophore with its surrounding) for the determination of chemicals[11-15]. In the present study, ISNAG-Fluorimeter[16-20] is the unique design was used for the determination of ketotifen fumarate depends on quenched of the continuous fluorescence of fluorescence azo dye which irradiated by two major low pressure mercury resonance line at 184.9 and 253.7nm and the measurement was detected by eight solar cells.

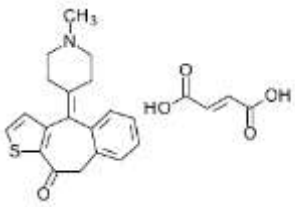


Figure 1: Chemical structure of ketotifen fumarate

CHEMICALS
The following stock solutions were prepared

- Ketotifen fumarate solution, 0.05 mol.L^{-1}(425.497g.mol^{-1}, SDI). Dissolve 2.1275g of $C_{23}H_{23}NO_5S$ in distilled water in a 100mL volumetric flask.
- Fluorescence azo dye (N-(4-Aryl diazenyl-p-phenol-1,8-naphthal imide)[21], 1mmol.L^{-1}(393g.mol^{-1}). Dissolve 0.0393g of $C_{24}H_{15}N_3O_3$ in ethanol in 100mL of volumetric flask.
- Buffer systems were prepared according to McIlvaine[22] $C_6H_8O_7$-Na_2HPO_4 (pH= 2.2-8.0):Citric acid 0.1 mol.L^{-1} (192.123g.mol^{-1}), dissolve 9.6062g of $C_6H_8O_7$ in distilled water of 500mL of volumetric flask
- Disodium hydrogen orthophosphate 0.2mol.L^{-1} (141.96g.mol^{-1}) , dissolve 14.196g of Na_2HPO_4 in distilled water of 500mL of volumetric flask

Apparatus
A new instrument of ISNAG-Fluorimeter is a genuine design which uses a long distance of 100mm for 2mm path length and low pressure mercury lamp (184.9 and 253.7nm) that capable of excited fluorescent molecules and then detected by 2x4 (each one consist of 25mm length x14mm width) solar cells of 410-1150nm (measure fluorescence emission on $2x90^0$, i.e: at both of 90^o angle). Manifold design

consist of: peristaltic pump (ISMATIC, type ISM 796, Switzerland), 6-port medium pressure injection valve (INDEX corporation, USA), potentiometric recorder (KOMPENSO Graph C-1032, Siemens, Germany 1-500volt).

METHODOLOGY

Using one line system (fig.2) designed for the determination of ketotifen fumarate via the use of continuous of fluorescence azo dye (0.07mmol.L^{-1}, which prepared at pH=6(12.63mL Na_2HPO_4-7.37mL citric acid) as carrier stream (2.0mL.min^{-1}) leading towards 6-port injection valve carrying movable segment (125 µL, 0.05 mmol.L^{-1}) that passes through ISNAG-Fluorimeter to measure quenched of the continuous fluorescence of organic azo dye. A proposed mechanism for the quenched of fluorescence occurred in the manifold system expressed in sketch number 1.

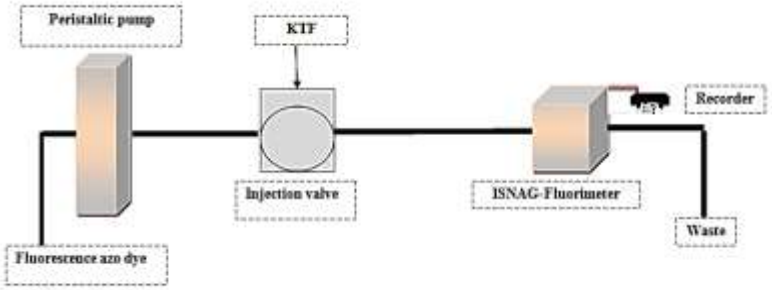
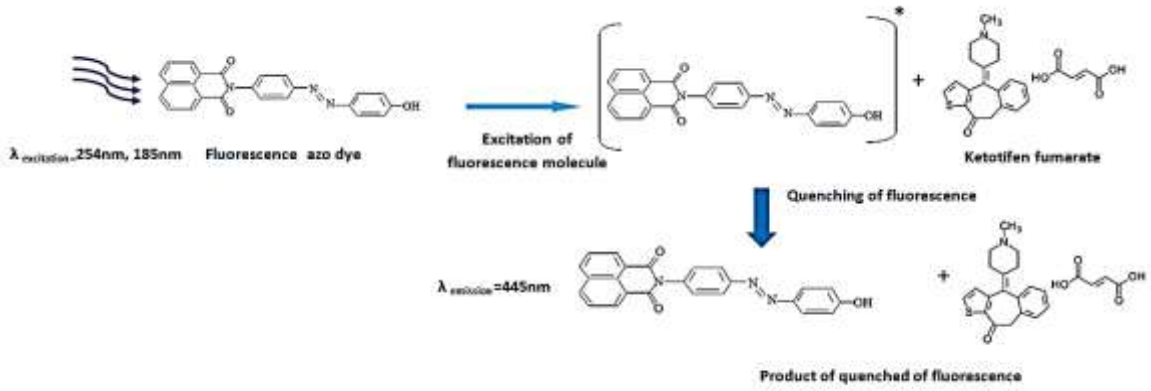


Figure2: Schematic diagram of manifold system for the determination of ketotifen fumarate



Sketch number1: Schematic representation of the proposed mechanism for the reaction between fluorescence dye molecules with ketotifen fumarate

RESULTS

Chemical Variables

Solutions of fluorescence azo dye ranged 0.01-0.3mmol.L^{-1} were prepared to study the effect of continuous fluorescence intensity. From table 1 and fig.3 it can be seen increase of continuous fluorescence with increase of fluorescence azo dye concentration also increased quenching response up to 0.07mmol.L^{-1}. Above 0.07 mmol.L^{-1} found that a decreased quenching response by KTF (0.05mmol.L^{-1}) due to external conversion between molecules through molecular collisions so it was found 0.07mmol.L^{-1} of fluorescence azo dye is most suitable to use throughout this work.

Table 1: Variation of azo dye concentration for determination of KTF

[Azo dye] mmol.L^{-1}	Continuous of fluorescence of azo dye (Average peak height) ($\bar{y}$i ,n=3,mV)	Quenched the fluorescence of azo dye $\bar{y}_{Qi}$ (n=3) mV	Repeatability of quenched fluorescence response (95% confidence level,α=0.05) $\bar{y}_{Qi}$ (mV)$\pm t_{0.05/2,n-1}$, $\sigma_{n-1}/\sqrt{n}$
0.01	198	80	80±2.09
0.03	282	100	100±1.32
0.05	395	128	128±1.67
0.07	982	168	168±2.29

0.1	981	160	160±2.16
0.3	980	160	160±1.81

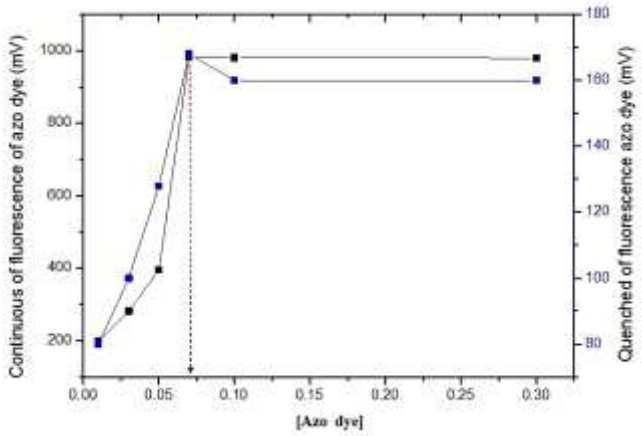


Figure 3: Variation of azo dye concentration for determination of KTF

PH variation

At a selected concentration of KFT (0.05mmol.L^{-1}) was used for a set of variable buffer solutions (pH 2.2-8.0) to study the influence of pH on the continuous and quenched fluorescence response. The results (table 2, fig.4) shows that an increase of continuous of fluorescence intensity with increase of buffer solution up to pH=6.0 in which that obtained a maximum of continuous fluorescence which lead to increase of the quenched of fluorescence by KTF while at more pH lead to decrease quenched of fluorescence due to deactivation of fluorescence emission.

Table 2: Effect the variation of pH on the continuous fluorescence intensity and quenched fluorescence response

Buffers		pH	Continuous of fluorescence of azo dye (Average peak height) ($\bar{y}i$,n=3,mV)	Quenched the fluorescence of azo dye $\bar{y}_{Qi}$ (n=3) mV	Repeatability of quenched fluorescence response (95% confidence level,α=0.05) $\bar{y}_{Qi}$ (mV)$\pm t_{0.05/2,n-1}$, $\sigma_{n-1}/\sqrt{n}$
Na_2HPO_4 (mL)	Citric acid (mL)				
0.40	19.60	2.2	208	98	98±2.06
4.11	15.89	3.0	520	158	158±1.66
7.71	12.29	4.0	982	170	170±1.62
10.30	9.70	5.0	1030	178	178±2.19
12.63	7.37	6.0	1080	201	201±2.26
16.47	3.53	7.0	1010	201	201±2.21
19.45	0.55	8.0	960	201	201±2.34

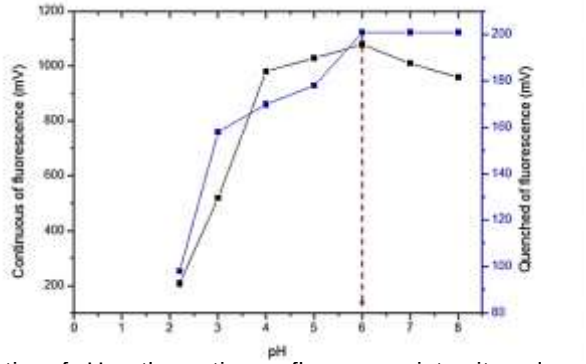


Figure 4: Effect the variation of pH on the continuous fluorescence intensity and quenched fluorescence response

Physical variables

Flow Rate

Using optimum concentration of fluorescence azo dye 0.07mmol.L^{-1} and 0.05mmol.L^{-1} of KFT, 100µL sample segment (open valve mode, 50sec) with flow rates ranged from 0.9-2.5mL.min^{-1}. From table3 and fig.5 it can be seen at 1.7-0.9mL.min^{-1} flow rate there is an increase in Δt_B of quenched fluorescence response and increase dispersion and dilution effect with decrease in S/N while at > 2.0mL.min^{-1} decrease of Δt_B and minimize of dilution effect and analysis time. Therefore, the best flow rate 2.0mL.min^{1}.

Table 3: Change of quenched fluorescence response at different flow rate

Flow rate (mL.min^{-1})	Quenched the fluorescence of azo dye $\bar{y}_{Qi}$ (n=3) mV	Repeatability of quenched fluorescence response (95% confidence level,α=0.05) $\bar{y}_{Qi}$ (mV)$\pm t_{0.05/2,n-1}$, $\sigma_{n-1}/\sqrt{n}$	Time lapse (sec)
0.9	128	128±2.46	90
1.2	193	193±2.16	80
1.5	201	201±1.84	60
1.7	210	210±2.21	40
2.0	250	250±1.32	36
2.3	210	210±1.04	30
2.5	179	179±1.27	28

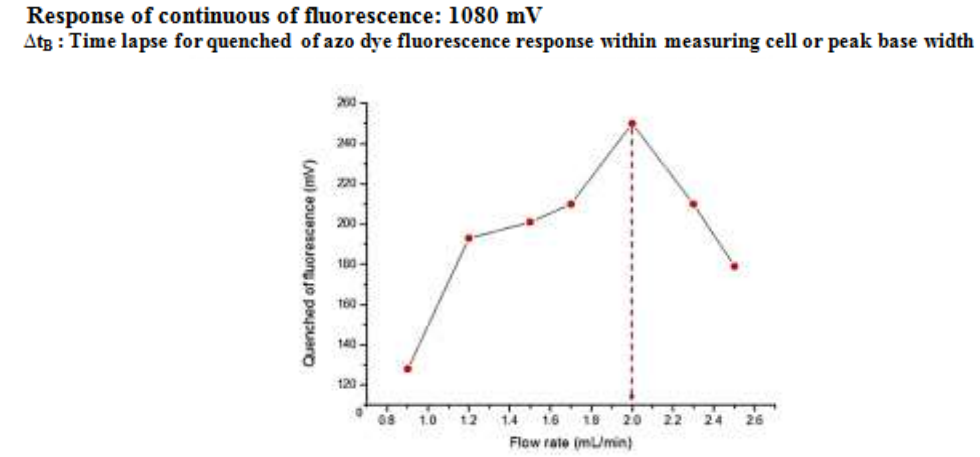


Figure 5: Effect the change of flow rate for determination of KTF

Sample volume

In order to optimization of sample volume, variable loops (50-250µL) were used with open valve mode. Table 4 shows that an increase of sample segment up to 125µL lead to increase in quenched fluorescence response. When using >125µL it gave a decrease of response height with continuation of the increase of Δt_B (peak base width) which might be probably attributed to increases the time duration of KTF segment in front of eight solar cells as a detector. Therefore, 125µL was most satisfactory.

Table 4: The change of quenched fluorescence response at different sample volume

Sample volume (µL)	Quenched the fluorescence of azo dye $\bar{y}_{Qi}$ (n=3) mV	Repeatability of quenched fluorescence response (95% confidence level,α=0.05) $\bar{y}_{Qi}$ (mV)$\pm t_{0.05/2,n-1}$, $\sigma_{n-1}/\sqrt{n}$	Time lapse (sec)
50	107	107±4.34	25
75	145	145±3.88	30
100	255	255±1.61	36
125	388	388±1.34	38
200	300	300±2.41	65

250	305	305±3.28	70

Response of continuous of fluorescence: 1085 mV

Δt_b : Time lapse for quenched of azo dye fluorescence response within measuring cell or peak base width

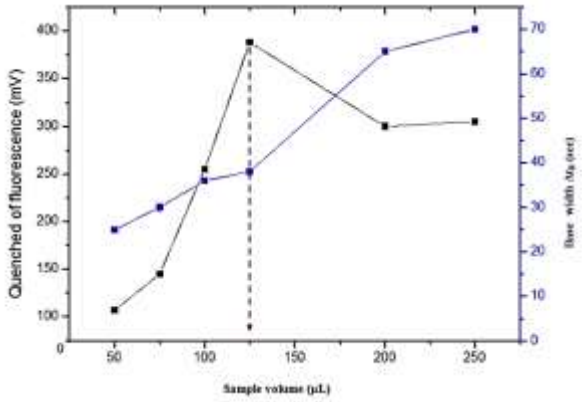


Figure 6: Relation between quenched fluorescence response, sample volume and peak base width

Purge time

Using all parameters that were achieved above, purge time was studied by dividing the KTF segment (sample segment) into portions depend on time factor (variable time 10-50 sec) that certain part of movable segment will be enter into carrier stream. Table 5 shows an increase in quenched fluorescence response with increase purge time up to 40sec followed by a littile decrease from quenched response with open valve mode (50sec), therefore 40sec was chosen as a better choice to insure a complete purge of the analyte segment without any dispersion and restriction of response can be obtained.

Table 5: Effect variation of purge time for determination of KTF

Purge time (sec)	Quenched the fluorescence of azo dye $\bar{y}_{Qi}$ (n=3) mV	Repeatability of quenched fluorescence response (95% confidence level,α=0.05) $\bar{y}_{Qi}$ (mV)$\pm t_{0.05/2,n-1}$, $\sigma_{n-1}/\sqrt{n}$
10	210	210±2.36
20	258	258±1.89
30	289	289±2.06
35	354	354±1.39
40	395	395±1.42
Open valve(50)	388	388±1.22

Response of continuous of fluorescence: 1083 mV

Preparation of calibration Curve and D.L

Solutions containing various KTF concentrations (0.01-10mmol.L^{-1}) were prepared under the parameters established above using continuous flow injection manifold system coupled with ISNAG-fluorimeter to measured quenched of the continuous fluorescence of fluorescence azo dye. Regression analysis gave the following equation: 361.67±47.39+85.506±11.03 [KTF] mMol.L^{-1}, with a r= 0.9837 (correlation coefficient), r^2 = 0.9676 (coefficient of determination) and R^2% = 96.76 (linearity percentage) as tabulated in table 6. Two different methods for the calculated of detection limit was used. First one depend on successive dilution of low concentration of KTF used of dynamic range of calibration graph and the second used the numeric value of slope (table 6).

Table 6: Sum up of calibration graph and detection limit results for the determination of ketotifen fumarate using

Range of ketotifen	$\hat{Y}_{(mV)}=a\pm s_a t+b\pm s_b t$ ketotifen fumarate	r	t_{tab} at 95%,n-		Detection limit(D.L) (µg/125µL)

fumarate mmol.L^{-1} (n= 12)	mmol.L^{-1} at confidence level 95%,n-2	r^2 R^2%	2	$t_{cal} = \frac{\|r\|\sqrt{n-2}}{\sqrt{1-r^2}}$	Practically based on the gradual dilution of the minimum concentration (0.008mmol.L^{-1})	Theoretical based on slope
0.01-10	361.67±47.39+85.506±11.03[KTF]mMol.L^{-1}	0.9837 0.9676 96.76	2.228<<17.28		0.425	9.237

[X]=KTF mmol.L^{-1}, $\hat{Y}$=estimate value , r = correlation coefficient
R^2% = Linearity percentage, r^2= coefficient of determination (C.O.D)
X= value of L.O.D. based on slope , S_B= standard deviation of blank repeated for 13 times
Limit of detection (L.O.D) and Repeatability

Analysis of Pharmaceutical Preparation

ISNAG-fluorimeter coupled with continuous flow injection system was used for the analysis of ketotifen fumarate in three types of drug manufactures (table 7). The quenched fluorescence response of KTF (1mmol.L^{-1}, 0.01064 g) was measured by traken 1mL to each volumetric flask (10mL capacity), followed by gradual volumes of standard solution of KTF (0.0,0.2,0.25,0.3,0.35mL from 50mmol.L^{-1}) to obtain 0.0-1.75mmol.L^{-1}) .

Table 7 shows the treatment of data using individual t-test to compare between individual mean with official quoted value (reference value=1mg) and can be noticed from the results obtained that any calculated t-value<4.303 therefore no significant difference found at 95% confidence level.

Table 7: Summary of practical content, equation of standard addition and efficiency for the determination of ketotifen fumarate

Sample No.	Commercial name content and company country	Confidence interval for the average weight $\overline{Wi}$±1.96 $\sigma_{n-1}/\sqrt{n}$ at 95% (g)	Sample weight equivalent to 0.01064 g (1mmol.L^{-1}) of the active ingredient (g)	Theoretical content of the active ingredient at 95% (mg)	Equation of standard addition at 95% for n-2 $\hat{Y}_{(mV)}$=a±s_at+b±s_bt r,r^2,R^2%	Practical content $\overline{Wi}$±4.303$\sigma_{n-1}/\sqrt{n}$ (mg) for (n=3), at 95% W_i(g) Efficiency of determination (Rec. %)	Individual comparison (X -μ) $\sqrt{n}/\sigma_{n-1}$ ISNAG fluorimeter- CFI with Quoted value t 0.05/2 ,2=4.303
1	Asmafort Julphar 1mg UAE	0.1236±0.0009	1.3151	1±0.0073	31.63±42.28+289.97±33.69 0.9980,0.9960,99.60%	1.090±0.243 0.0116 109	1.591<<4.303
2	Zaditen 1mg Switzerland	0.1313±0.0078	1.3970	1±0.0594	24.16±50.73+239.12±40.42 0.9958,0.9916,99.16%	1.015±0.1963 0.0108 101.5	0.329<<4.303
3	Help 1mg Greece	0.1208±0.0083	1.2853	1±0.0687	40.49±6.05+377.37±4.82 0.9999,0.9998,99.98%	1.071±0.211 0.0114 107.1	1.447<<4.303

$t_{0.025,\infty}$ = 1.96 at 95 % , σ_{n-1}:Difference standard deviation , n= no. of sample =3

CONCLUSION

The new instrument was used for the determination of ketotifen fumarate by irradiation using mercury low pressure lamp: 184.9nm and 253.7nm which capable to irradiate the flow cell and the fluorescenct light can be seen by the detector extended for 100mm on each side of the flow cell. The method was depend on quenched of fluorescence continuous response by ketotifen fumarate molecules in pure and three different dugs. A comparison was made using individual t-test between quoted value and practical value and its found that there was no significant difference between two values at 95% confidence (α=0.05).

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REFERENCES

1. British pharmacopoeia. 2012. 7th edition. The Stationery office, Londone.
2. Jack V , Richard C, Leonard P, Barry M, Nareed B and Mark B.2003. Efficacy and safety of ketotifen fumarate 0.025% in the conjunctival antigen challenge model of ocular allergic conjunctivitis.*American journal of Ophthalmology*, 136(6),p.1097.
3. Jafar E, Aida A, Ali M, Hamed H, Vahid P, Yalda R and Amir A.2018. Formulation, characterization and cytotoxicity evaluation of ketotifen loaded nanostructured lipid carriers. *Journal of Drug Delivery Science and Technology*,46,p.268.
4. Zvonimira M, Josip K, Hrvje H,Mladen Ž, Franjo K and Zlatko M.1984.Ketotifen. *Analytical Profiles of Drug Substances*, 13,p.239.
5. El-Kousy N and Bebawy L.1999.Determination of some antihistaminic drugs by atomic absorption spectrometry and colorimetric methods. *Journal of Pharmaceutical and Biomedical Analysis*, 20(4),p.671.
6. Khater M, Issa Y and Mohammed S.2009. Flow injection determination of ketotifen fumarate using PVC membrane selective electrodes. *Bioelectrochemistry*, 77(1),p.53.
7. Ali M, Mehrgan G, Mahdieh M, Mohsen K and Iraj E.2015. Simple chemiluminescence determination of ketotifen using tris(1,10 phenanthroline)ruthenium(II)-Ce(IV) system. *Luminescence*,30(7),p.1094.
8. Min Z, Yu-Jie L, Yong-Jun M, Wie-Feng W, Juan M and Hui C.2011.Determination of ketotifen fumarate by capillary electrophoresis with tris(2,2′ - bipyridyl)ruthenium(II) electrochemiluminescence detection. *The journal of Biological and Chemical Luminescence*, 26(5),p.319.
9. Nagam S and Manhl H.2016. Quantitative determination of ketotifen fumarate in the pure form and tablets via use of turbidity by reaction with Cerium(IV) sulfate using Ayah 6SX1-T-1D CFI analyser. *International journal of research in pharmacy and chemistry*, 6(3),p.411.
10. Nagam S and Hayder Q.2019. New fluorometric method for the determination of ketotifen fumarate using continuous flow injection analysis via ISNAG-fluorimeter. *Baghdad Science Journal*, 16(2),p.253.
11. Rubio L, Sanllorente S, Sarabia L and Ortiz M.2019. Determination of cochineal and erythrosine in cherries in syrup in the presence of quenching effect by means of excitation-emission fluorescence data and three-way PARAFAC decomposition. *Talanta*, 196(1),p.153.
12. Xiangling R, Jiejie G, Xianwei M, Xiaozhong Q, Jun R and Fangaiong T.2016. Sensitive detection of dopamine and quinone drugs based on the quenching of the fluorescence of carbon dots. *Science Bulletin*, 61(20),p.1615.
13. Haicony S, Shiwei Z, Qiangqiang F, Wei X, Shifeng W, Shiting X, Meng X, Hongfen B and Yong T.2017. A membrane-based fluorescence-quenching immunochromatographic sensor for the rapid detection of tetrodotoxin. *Food Control*, 81,p.101.
14. Yusheng Y, Liu Y, Shaopu L, Jidong Y, Hui Z, Jingjing Y and Xiaoli H.2017. Enzyme-catalyzed Michael addition for the synthesis of warfarin and its determination via fluorescence quenching of L-tryptophan. *Spectrochimica Acta Part A:Molecular and Biomolecular Spectroscopy*, 176(5),p.183.
15. Jing T,Shaopu L, Zhongfang L, Jidong Y, Jinghui Z, Man Q and Xiaoli H.2014. Fluorescence quenching and spectrophotometric methods for the determination of daunorubicin with meso-tera (4-sulphophenyl) porphyrin as probe. *Spectrochimica Acta Part A:Molecular and Biomolecular Spectroscopy*, 120(24),p.7.
16. Issam M and Nagam S.2014.ISNAG fluorimeter for fluorescence measurement at the visible region of electromagnetic radiation using mercury tube lamp as a source for irradiation for continuous flow analysis using solar cells, Patent, H01J61/00.
17. Nagam S and Hayder Q.2018. Assessment of ISNAG fluorimeter (Total fluorescence measurements at+
18. 90° & - 90° using four solar cell on each side for 100mm distance at 2mm path length) with well-known fluorescent molecules via CFIA. *Iraqi Journal of Science*, 59,p.240.
19. Nagam S and Hussein F.2018.Irradiation of formed reaction product (precipitate) with a short wave lengths (184.9&253.7nm) as a new mode for measurement of scattered light using multi solar cells to collect improved analytical signals for continuous flow injection analysis. Thesis.PhD. University of Baghdad.Iraq.
20. Nagam S and Hussein F.2018. Newly Developed Method for Determination Indomethacin using Potassium Hexacyanoferrate (III) by using ISNAG-Fluorimeter Homemade via CFIA. *Journal of global pharma technology*, 10(6),p.447.
21. Nagam S and Hussein F.2019. Determination of Mefenamic Acid using 8-hydroxy quinoline as a Precipitating Agent and Low Pressure Mercury Lamp (184.9 & 253.7 nm) as a Source of Irradiation using of ISNAG Continue Flow Fluorimeter. *Journal of global pharma technology*, 11(3),p.333.
22. Suaad M and Mohammed G.2019.Synthesis and characterization of some new hetrocyclic derivatives from 1,8-Naphthalic anhydride and studing their possible applications as antimicrobial agents, photo physics or dyestuffs. Thesis.PhD. University of Baghdad.Iraq.
23. McIlvaine T.1991. A buffer solution for colorimetric comparison. *Journal of Biological Chemistry*, 49,p.6