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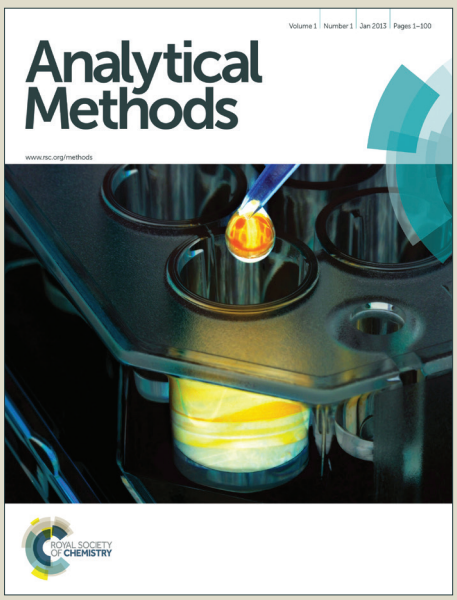

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A Rapid and Highly Sensitive Fluorimetric Method for the Determination of Meloxicam Using Uranyl Acetate

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Abstract

In this work, the fluorescence of a metal ion (UO_2^{2+}) was exploited for the determination of meloxicam. The fluorescence quenching values of Uranyl Acetate (UA) showed an excellent linear relationship to the concentration of meloxicam (MLX) over the range of 0.015 ~ 7.5 μg mL^{-1}. The detection limit (3σ) was 4.49 ng mL^{-1}, which was lower than or comparable to most of the previously reported methods. The proposed method was applied satisfactorily to the assay of MLX in tablet and capsule samples. The optimum reaction conditions, influencing factors and effects of coexisting substances have been investigated. And the absorption spectrum, quenching constants (K_q) and Stern-Volmer plots confirmed that this was a dynamic quenching process.

Keywords: meloxicam; uranyl acetate; fluorescence dynamic quenching

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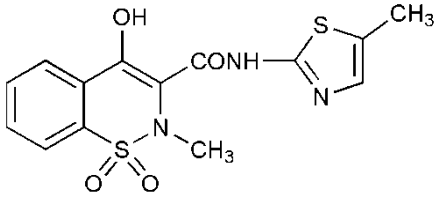
INTRODUCTION

Meloxicam(MLX) ,4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide (Scheme 1) is a potent non-steroidal anti-inflammatory drug (NSAID) in the group of enolic acids found to preferentially inhibit cyclo-oxygenase-2 (COX-2). It is used for the treatment of rheumatoid arthritis, osteoarthritis and other joint diseases.[1] Meloxicam is widely used due to its advantages like high anti-inflammatory analgesic activity, good tolerance, small side effects and so on.[2,3]



Scheme 1 The structure of meloxicam

Although uranium plays an important role in our life and production, it is a common radioactive pollution source. So the studies about uranium in analytical application mainly focus on detecting content of uranium in environment.[4,5] However, there is few applications about compound of uranyl (UO_2^{2+}) in drug analysis used spectrophotometric method,[6] let alone reports on fluorescence probe to determine drug.

Different methods on the determination of MLX have been reported, such as high performance liquid chromatography (HLPC),[7-9] spectrophotometry,[10] voltammetry,[11] enhanced spectrofluorimetric method,[12] capillary zone electrophoresis,[13] chemiluminescence[14] and electrochemical method.[15]

Nevertheless, among those methods, some have low sensitivity and poor selectivity, while some require expensive apparatus and harsh conditions, and containing several time-consuming

steps during manipulation operation like heating or extracting. Hence, it is of great significance to further develop a simple and quick method of molecular spectrometry to determine MLX with high sensitivity and good selectivity.

Uranyl acetate (UA) emits intense green fluorescence, and its maximum excitation wavelength (λ_{ex}) and maximum emission wavelength (λ_{em}) are located at 280 and 516 nm in pH 7.05 – 7.25 weak alkaline medium. The interaction of UA and MLX can lead to fluorescence quenching of UA at room temperature without heating, catalyst or waiting for a long time, the fluorescence quenching values (ΔF) are linearly correlated to the concentration of MLX within certain limits. So, a new fluorimetric method using UA as probe to detect MLX is proposed in this paper, and this method can be applied accurately to determine meloxicam in tablet and capsule samples.

EXPERIMENTAL

Apparatus and reagents

A Hitachi F-2500 spectrofluorophotometer (Tokyo, Japan) was used for recording fluorescence spectra and measuring fluorescence intensities at a given wavelength using a 1 cm path length. A UV-Vis 2450 spectrophotometer (Tokyo, Japan) was used for recording absorption spectra and measuring the absorbance. A pHS-3D pH meter (Shanghai Scientific Instruments Company, China) was used to measure the pH values. A KQ-250B Ultrasonic instrument acoustic cleaner (Kunshan Ultrasonic Instruments Company, China) was used to help dissolving solids.

The stock solution of MLX (500.0 μg mL^{-1}, national institutes for food and drug control) and UA (1.0×10^{-2} mol L^{-1}, Czech import) were prepared and kept at 4℃, respectively. Working solutions were freshly prepared by diluting the corresponding stock solutions. Tris-HCl buffer

solutions with different pH were prepared by mixture 0.1 mol L^{-1} Tris and 0.1 mol L^{-1} HCl in proportion. The pH was adjusted with a pH meter. All other reagents were analytical reagent grade and used without further purification. Doubly distilled water was used throughout.

General procedure

1.0 mL of pH 7.2 Tris-HCl buffer solutions, 2.0 mL of UA (1.0×10^{-3} mol L^{-1}) and appropriate amount of MLX solution (30.0 μg mL^{-1}) are added into a 10.0 mL calibrated flask. The mixture are then diluted to the mark and mixed thoroughly. After 3 min, the fluorescence spectra of system are recorded with scanning at λ_{ex} / λ_{em} =280 /516 nm. The change intensities of fluorescence is denoted as $\Delta F=F_0 - F$, (F and F_0 are fluorescence intensities of system and reagent blank).

RESULTS AND DISCUSSION

Fluorescence spectrum

UA has strong fluorescence, whose maximum excitation wavelength (λ_{ex}) and maximum emission wavelength (λ_{em}) are located at 280 nm and 516 nm, respectively. while MLX has no fluorescence. As UA interacted with MLX, the characteristic for fluorescence spectra of UA scarcely changed, but its fluorescence intensity was markedly quenched as showed in Fig.1. In a certain range, ΔF is direct proportion to the concentration of MLX, so fluorescence method can be used for the determination of MLX.

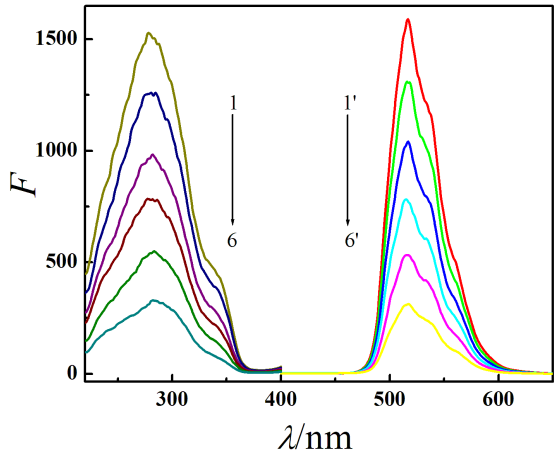


Fig. 1 The excitation and emission spectra of UA and UA-MLX system. 1~6: excitation spectra; 1'~6': emission spectra; 1 and 1': UA ; 2~6 and 2'~6': UA-MLX system, c_{UA}= 2.0 × 10^{-4} mol L^{-1} ; c_{MLX}(from 2,2' to 6,6') = 1.5, 3.0, 4.5, 6.0, 7.5 μg mL^{-1}; Tris-HCl buffer, pH 7.2.

Effect of the acidity

The influences of different buffer solution on fluorescence intensity of the reaction are tested with BR, HAc-NaAc, Na_2HPO_4-citric acid, potassium acid phthalate-NaOH and Tris-HCl buffer solutions. The results showed that the fluorescence quenching intensity of UA-MLX system was the highest in Tris-HCl buffer solution. Therefore, Tris-HCl buffer solution was selected to control the pH of solution. And the fluorescence quenching intensity reached the maximum in the range of pH 7.05 − 7.25 (as shown in Fig.2). Thus, pH 7.2 Tris-HCl buffer solution was selected and the amount of buffer solution was tested as 1.0 mL.

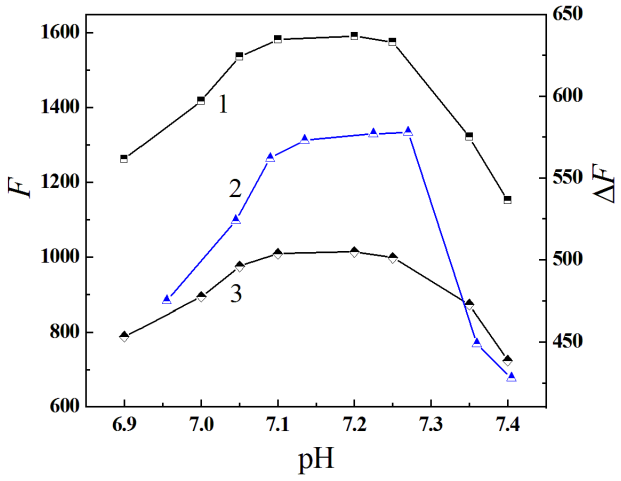


Fig. 2 The effect of the pH on Fluorescence. curve 1: the fluorescence intensity of UA; curve 2: the fluorescence quenching values (ΔF) of UA in the presence of MLX; curve 3: the fluorescence intensity of UA-MLX system; c_{UA}= 2.0×10^{-4} mol L^{-1}; c_{MLX}= 3.0 μg mL^{-1}.

Effect of UA concentration

At pH 7.2 Tris-HCl buffer solution, the influence of the concentration of UA on the fluorescence quenching intensity of UA-MLX system was investigated when the concentration of MLX was 3.0 μg mL^{-1}. The results showed that the fluorescence quenching intensity enhanced with the increasing of UA concentration in a range of low concentration and then decreased with the increasing of UA concentration in a range of high concentration. And the appropriate working concentration of UA was $1.5\times10^{-4} \sim 3.0\times10^{-4}$ mol L^{-1}.

When the concentration of UA was lower than the above concentration, the reaction of UA with MLX was incomplete and could lead to ΔF lower. If the amount of UA increases the fluorescence intensity decreases because of inner filter effect and against determination MLX (showed in Fig.3). Consequently, 2.0×10^{-4} mol L^{-1} was chose as suitable UA concentration.

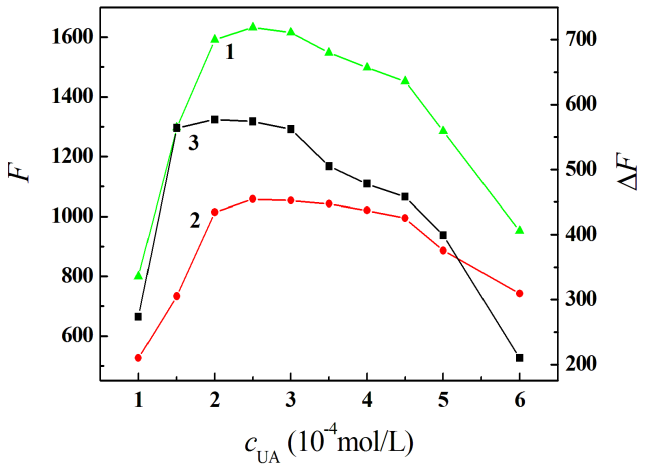


Fig. 3 The effect of the concentration of UA on Fluorescence intensity. curve 1: the fluorescence intensity of UA; curve 2: the fluorescence intensity of UA-MLX system; curve 3: the fluorescence quenching values (ΔF) of UA in the presence of MLX; c_{MLX}= 3.0 μg mL^{-1}, Tris-HCl buffer, pH 7.2.

Reaction speed and the stability of fluorescence intensity

At room temperature, the reaction was finished in 3 minutes and fluorescence intensity could remain constant for 4 hours at least. Experiments were carried out after 3 minutes (showed in Fig.4).

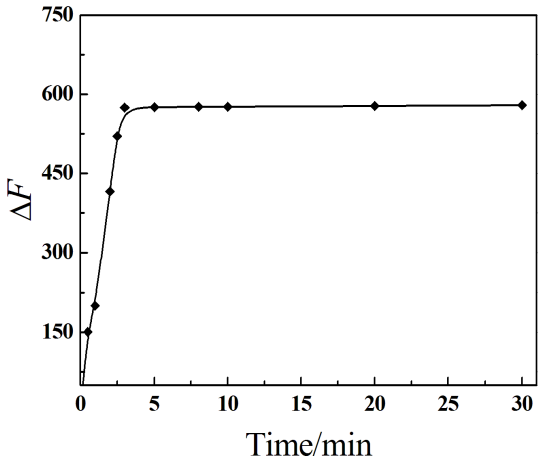


Fig. 4 The effect of reaction time. c_{UA}= 2.0 × 10^{-4} mol L^{-1}; c_{MLX}= 3.0 μg mL^{-1}; Tris-HCl buffer, pH 7.2.

The mechanism of quenching of UA fluorescence by MLX

Fluorescence quenching can proceed via different mechanisms, usually classified as dynamic quenching and static quenching.[16] The dynamic quenching is mainly caused by the fluorescence substance collision with quencher, and result in the decreased of the quantum yield and fluorescence intensity. A non-fluorescent compound, formed by quencher and fluorescence substance, led to the static quenching.

Dynamic and static quenching can be distinguished, these dependence on different temperature of binding constants and viscosity, or preferably by lifetime measurements. The quenching constants decrease with increasing temperature for static quenching, while the reverse effect is for dynamic quenching. Assuming that the process belongs to dynamic quenching, we analyzed the fluorescence data at different temperatures with the well-known Stern-Volmer equation:[17]

$$F_0/F=1+K_q\tau_0[Q]=1+K_{sv}[Q]$$

where F and F_0 are the fluorescence intensities of UA in the presence of and in the absence of the quencher, respectively; K_{sv} is the dynamic quenching constant; K_q is the quenching rate constant, $K_q=K_{sv}/\tau_0$; τ_0 is the average lifetime of the molecule without MLX (2.578 μs);[18] [Q] is the UA concentration. The Stern-Volmer plots of the UA fluorescence quenching by MLX at three different temperatures are displayed in Fig.5. Some parameters for Stern-Volmer plots are listed in Table 1. In this experiment condition, quenching constant K_{sv} increased as temperature rose (Fig. 5 and Table 1), this is a remarkable characteristic of dynamic quenching. In addition, according to $K_q=K_{sv}/\tau_0$, the quenching rate constant K_q are 1.404×10^{10}、1.780×10^{10} and 2.227×10^{10} L $(mol\cdot s)^{-1}$ between 283 and 308 K (Table 1). As known, the biggest K_q of collisional quenching is $(1.0 \sim 2.0)\times10^{10}$ L $(mol\cdot s)^{-1}$.[19,20] K_q values of this experiment are closed to it. The results indicated that the reaction of MLX with UA is a single dynamic quenching

process.

Table 1 The parameters of Stern-Volmer equation

Temperature T/K	Stern-Volmer equation ([Q]/μmol L^{-1})	Correlation coefficient (r)	Ksv L mol^{-1}	Kq L (mol·s)$^{-1}$
283K	F_0/F=1.0+3.62×10^{-2}[Q]	0.9985	3.62×10^4	1.404×10^{10}
298K	F_0/F=1.0+4.59×10^{-2}[Q]	0.9972	4.59×10^4	1.780×10^{10}
308K	F_0/F=1.0+5.74×10^{-2}[Q]	0.9989	5.74×10^4	2.227×10^{10}

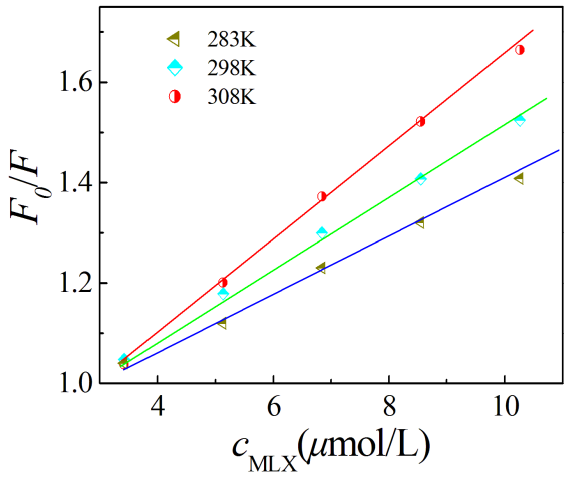


Fig.5 Stern-Volmer Curves for UA-MLX system at three different temperatures. c_{UA}= 2.0×10^{-4} mol L^{-1} ; c_{MLX}=3.42, 5.13, 6.84, 8.55, 10.26 μmol L^{-1} ; Tris-HCl buffer, pH=7.2.

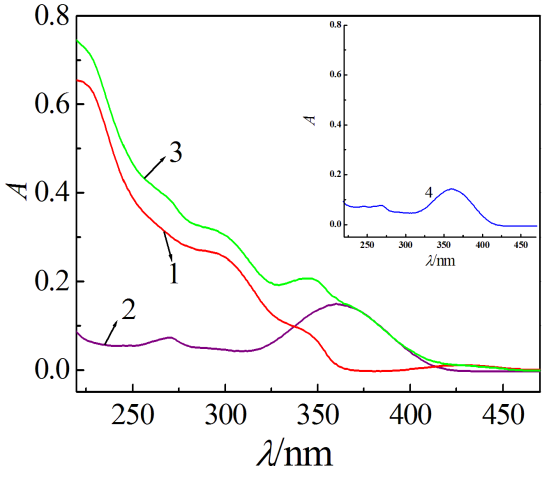


Fig.6 The absorption spectra of UA, MLX and UA-MLX system. 1. UA； 2. MLX; 3, 4. UA-MLX system; 1~3. against a water blank; 4. against a reagent blank; c_{MLX}=3.0 μg mL^{-1}; c_{UA}=2.0×10^{-4} mol L^{-1}; Tris-HCl buffer, pH=7.2.

UV–visible absorption studies

The absorption spectra of this experiment were recorded and presented in Fig. 6. As all we known that UV–visible absorption measurement is a very simple method and applicable to explore the structural change and formation of a complex.[21] The absorbance of UA-MLX system almost equals the sum of that of the MLX and UA. Furthermore, the absorbance and location of MLX against water blank and UA-MLX system against a reagent blank almost have no change. It indicated that the fluorescence quenching of UA is mainly caused by collision between UA and MLX. In other words, it supported the conclusion that the dynamic quenching exists in the interaction of UA and MLX.

Sensitivity and selectivity of the method

Under the optimum experimental conditions, the fluorescence intensities of UA who react with the different concentrations of MLX are measured at 280 / 516 nm after 3 minutes. The calibration graphs are gained by ΔF being plotted against the concentrations of MLX. The result showed that ΔF is proportional to the concentration of MLX for UA-MLX system at the range of 0.015 ~7.5 μg mL^{-1}. Equation of linear regression of standard curve is ΔF=44.3+167.08 c (c: μg mL^{-1}), the relative coefficient is 0.9993 (as shown in Fig.7) and the detection limit is 4.49 ng mL^{-1}. It can be seen that fluorescence quenching method for the determination of MLX used UA as fluorescence probe has high sensitivities. The proposed method offer improvements in terms of sensitivity and response times, as compared to some previously reported methods (Table 2). So this method is simple, fast, and more advantageous to the trace determination of MLX concentration.

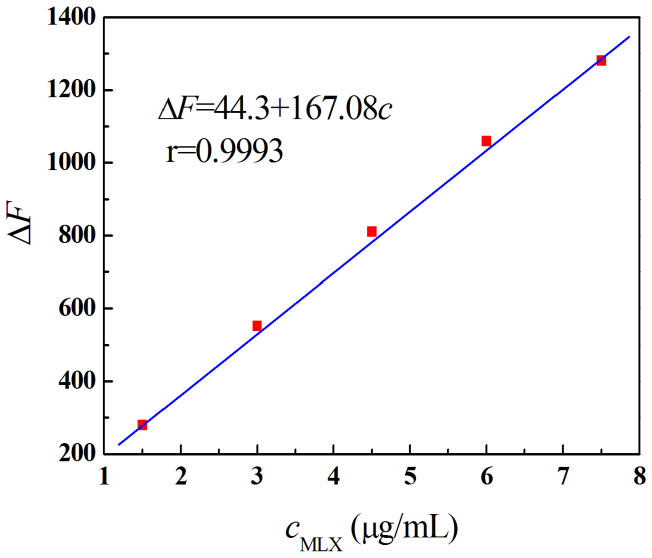


Fig.7 The standard curve of UA-MLX system

c_{UA}= 2.0 × 10^{-4} mol L^{-1} ; c_{MLX} = 1.5, 3.0, 4.5, 6.0, 7.5 μg mL^{-1}; Tris-HCl buffer, pH 7.2.

Table 2 Comparison of the sensitivities of fluorimetric method with some other methods for the determination of meloxicam

Method	Linearity	Detection limit	Medium	Reaction time
LC[7]	50−1500 ng mL^{-1}	50 ng mL^{-1}	acetonitrile	6 min
HPLC[8]	100−500 μg mL^{-1}	3.65 μg mL^{-1}	methanol	13.83 min
HPLC-UV[9]	10–2400 ng mL^{-1}	10 ng mL^{-1}	acetonitrile/K_2HPO_4	11.6 min
Direct Spectrophotometric[10]	2.0-10.0 μg mL^{-1}	0.11 μg mL^{-1}	ethanolic hydrochloric acid	Not given
Indirect Spectrophotometric[10]	4.0–12.0 μg mL^{-1}	0.33 μg mL^{-1}	Safranin T	Not given
Single sweep oscillopolarography[11]	(9.0-600.0)×10^{-8} mol L^{-1}	3.0×10^{-8} mol L^{-1}	HAc-NaAc/ethyl alcohol	Not given
Capillary zone electrophoresis[13]	0.5-150.0 μg mL^{-1}	0.3 μg mL^{-1}	MeOH	Not given
Chemiluminescence[14]	(2.2-280.0)×10^{-7} mol L^{-1}	7.7×10^{-8} mol L^{-1}	N-bromosuccinimide	Not given
Electrochemical[15]	(1.0-500.0)×10^{-8} mol L^{-1}	2.9×10^{-9} mol L^{-1}	Britton-Robinson buffer	Not given
Present work	0.015-7.5 μg mL^{-1}	4.49 ng mL^{-1}	Tris-HCl	3 min

The interference effects of some coexisting substances on the determination of MLX are investigated according to the experimental procedure and the results are showed in Table 3. It

can be seen that when the relative error is lower than ±5%, and the concentration of the MLX is 3.0 mg L^{-1}, much common metal ions, acid radical anions, inorganic anions, other sugars, proteins and amino acids have less effect on the method. Therefore, the method for determination of MLX has good selectivity.

Table 3 The effects of coexistence substances (c_{MLX} = 3.0 mg L^{-1})

Coexistent species	Concentration /mg L^{-1}	Relative Error (%)	Coexistent species	Concentration /mg L^{-1}	Relative Error (%)
sucrose	200.0	2.3	$MnSO_4$	80.0	-3.2
maltose	200.0	2.5	$(NH_4)_2S$	80.0	4.3
glucose	200.0	-4.1	$CaCl_2$	60.0	2.1
fructose	100.0	-4.4	$CoCl_2$	50.0	-3.7
starch	100.0	3.3	$Pb(NO_3)_2$	35.0	-4.3
carbamide	80.0	-1.5	KH_2PO_4	160.0	3.9
BSA	20.0	-4.9	NH_4Cl	160.0	-4.2
valine	200.0	-2.5	KCl	50.0	4.7
L-leucine	200.0	-4.7	NaCl	50.0	4.9
L-arginine	40.0	-4.8	$Mg(Ac)_2$	40.0	-1.6
L-proline	200.0	2.4	$ZnSO_4$	50.0	-2.3
L-glycine	200.0	-0.8	$CuSO_4$	10.0	-4.4
L-threonine	200.0	-2.7	$KAlSO_4$	30.0	-4.3

Analytical application

Ten tablets and capsules of MLX were weighed, ground and mixed, respectively. An amount of powder (equal to 7.5 mg of MLX) was dissolved in a small amount of 0.1 mol L^{-1} NaOH solution, filtered and the solution transferred into a 1000 mL calibrated flask and diluted

to the mark with water. 1.0 mL MLX solution of this tablets or capsules was pipetted into a 10 mL calibrated flask and MLX was detected via the fluorescence quenching method according to the general procedure, and the results are showed in Table 4.

It can be seen that fluorescence quenching method has a good repeatability for the determination of MLX in tablets and capsules, and relative standard deviation are between 2.3% and 3.4%. The method also has a good accuracy and the average recoveries are between 98.2% and 102.6% (Table 4). Therefore, this method can be applied to detect MLX in the tablets and capsules accurately and rapidly.

Table 4 Results for the determination of MLX in MLX tablet and capsule

Sample	Found/mark (mg/one tablet or capsule)	Added (mg/one tablet or capsule, n=5)	Total Found (mg/one tablet or capsule, n=5)	Recovery (%)	RSD (%)
Tablet 1	7.48/7.5	7.5	15.37	102.6	2.3
Tablet 2	7.48/7.5	26.3	34.26	101.4	3.1
Tablet 3	7.48/7.5	37.5	44.57	99.1	2.7
Capsule 1	7.47/7.5	7.5	15.13	101.1	2.4
Capsule 2	7.47/7.5	26.3	33.16	98.2	2.5
Capsule 3	7.47/7.5	37.5	44.23	98.4	3.4

CONCLUSIONS

In weak alkaline medium, UA can interact with MLX which can lead to the fluorescence quenching of UA. In a certain range, the ΔF was proportional to the concentration of MLX. In addition, this method was successfully applied to determine MLX in tablets and capsules with standard addition method. Therefore, a novel spectrofluorimetric method with high sensitivity, simplicity and quickness for determining MLX was established.

Furthermore, the fluorescence intensity analysis shows that the procedure is a dynamic

quenching process, which is also demonstrated by temperature, the Stern-Volmer plots and the change of absorption spectrum.

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REFERENCES

1. N. M. Davies and N. M. Skjodt, Clin. Pharmacokinet., 1999, 36(2), 115-126.
2. N. Ma, Y. Luo, Q. Meng, S. Zhong, Y. He, Chin. J. Mod. Appl. Pharm., **2000**, 17, 134.
3. H. Yu, Y. Fang, Chin. J. Pham. Anal., **2001**, 21, 124.
4. A. Rezaei, H. Khani, M. Masteri-Farahani, and M. K. Rofouei, Anal. Method., 2012, 4(12), 4107-4114.
5. Y. Yang, S. Xiao, Y. Zhang, L. Huang, and Q. Liu, Anal. Method., 2013, 5(18), 4785-4789.
6. J. Liu, C. Wang, X. Zhang, Y. Liu and W. Liu, Chin. J. Pharm., **1994**, 25, 272.
7. B. Dasandi, H. Saroj, Shivaprakash and K. M. Bhat, J. Pharm. Biomed. Anal., **2002**, 28, 999-1004.
8. N. H. Zawilla, M. A. Mohammad, N. M. El kousy and S. M. Ei-Moghazy Aly, J. Pharm. Biomed. Anal., **2003**, 32, 1135.
9. J. Bae, M. Kim, C. Jang and S. Lee, J. Chromatogr. B, **2007**, 859, 69.
10. M. E. Hassan, J. Pharm. Biomed. Anal., **2002**, 27, 771.
11. H. Huang, H. Gao and Y. Zeng, Chin. J. Anal. Chem., **2000**, 28, 1501.
12. J. Zhang, S. Chen, J. Chen, H. Ye and Y. Geng, Chin. J. Pharm. Anal., **2006**, 26, 1646.

13. E. Nemutlu and S. Kir, J. Pharm. Biomed. Anal., **2003**, 31, 393.

14. H. Liu, L. Zhang, Y. Hao, Q. Wang, P. He and Y. Fang, Anal. Chim. Acta, **2005**, 541, 187.

15. A. M. Beltagi, M. M. Ghoneim and A. Radi, J. Pharm. Biomed. Anal., **2002**, 27, 795.

16. G. Chen, X. Huang, B. Zheng, J. Xu, Z. Wang, Fluorescence Analysis, 2nd ed., Science Press: Beijing, 1990.

17. J. Xu and Z. Wang, Spectrofluorimetry, 3rd ed., Science Press: Beijing, 2006.

18. S. K. Das and B. N. Ganguly, J. Colloid. Interf. Sci., **1996**, 180, 377.

19. J. R. Lakawicz, Principles of Fluorescence Spectroscopy, 3rd ed., Springer-Verlag: Berlin Heidelberg, 2006.

20. Y. Ni, S. Wang and S. Kokot, Anal. Chim. Acta, **2010**, 663, 139.

21. X. Han, P. Mei, Y. Liu, Q. Xiao, F. Jiang and R. Li, Spectrochim. Acta. A, **2009**, 74, 781.