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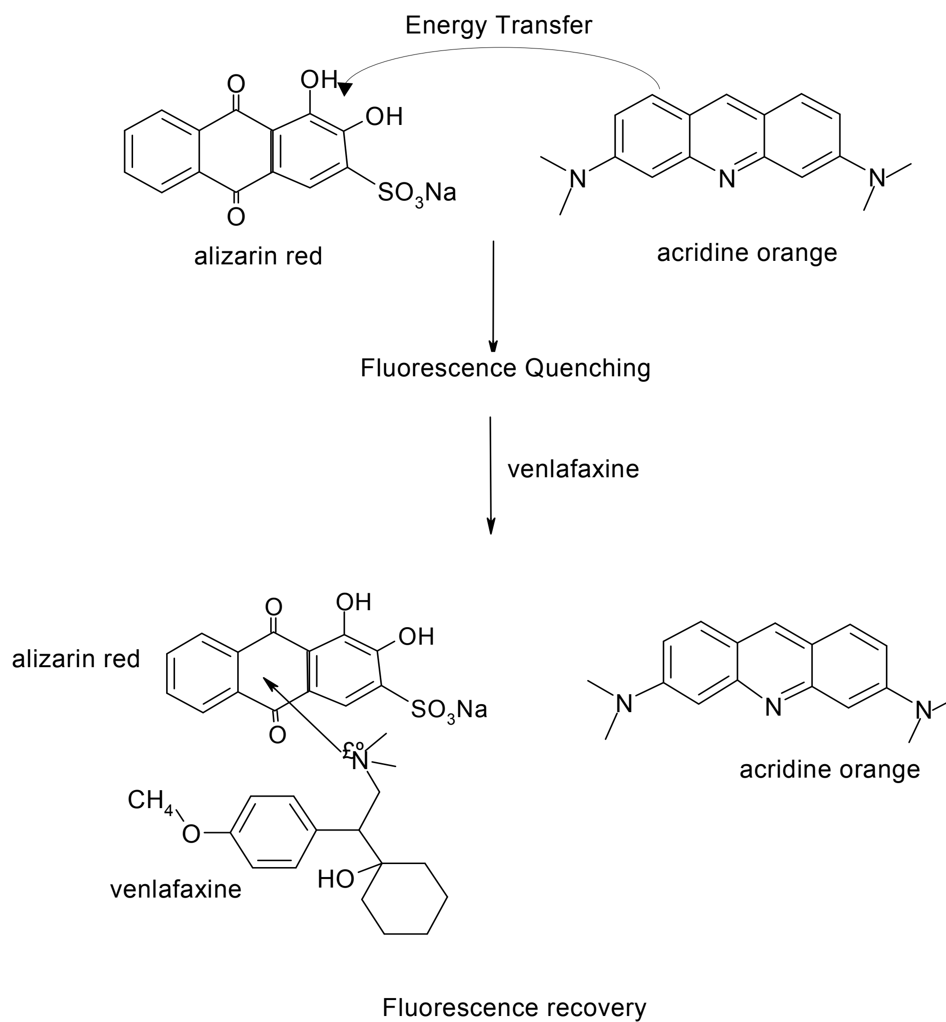

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1 # A Fluorescence Method for the Determination of Venlafaxine
2 Hydrochloride

3

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6

7 **Abstract:** In this study, fluorescence quenching of acridine orange was observed
8 when the effective energy transfer happened between alizarin red and acridine orange
9 under aqueous conditions with a pH of 6.8. However, the quenched fluorescence in
10 this aqueous solution could be partially recovered with the addition of an appropriate
11 amount of venlafaxine hydrochloride. And the recovery of fluorescence intensity had
12 a good linear relationship with the amount of added venlafaxine hydrochloride. Based
13 on this, a novel fluorescence recovery method for the determination of venlafaxine
14 hydrochloride was established. The experimental results showed that under optimal
15 conditions, the linear range of this method was 3.49–31.4 $mg \cdot L^{-1}$, the detection limit
16 was 1.65 $mg \cdot L^{-1}$, and the precision was 1.16%. The method was used for the
17 determination of venlafaxine hydrochloride in sustained-release tablets and capsules
18 with satisfactory results, and the recoveries were in the range of 95.5%–105%.

19 **Keywords:** venlafaxine hydrochloride, fluorescence recovery, acridine orange,
20 alizarin red

21

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22 **1 Introduction**

23 Venlafaxine hydrochloride (VLX), a second-generation non-tricyclic antidepressant,
24 was used clinically to treat depression and certain types of anxiety disorders with mild
25 side effect in recent years. Currently, some methods for the determination of
26 venlafaxine hydrochloride have been reported, such as high performance liquid
27 chromatography (HPLC) [1–12], liquid chromatography-mass spectrometry [13–19],
28 visible spectrophotometry [20, 21], ultraviolet spectrophotometry [22, 23], electrical
29 analysis [24–26], and charge-transfer spectrophotometry [27] and so on. These
30 methods all have its relative merits, but there are still some unsatisfactory aspects. For
31 instance, high performance liquid chromatography and liquid chromatography-mass
32 spectrometry methods require complicated sample pretreatment procedure, expensive
33 instrument and high operative expenses. Spectrophotometry and electroanalysis
34 methods have low selectivity and were easy to be interfered by the excipients.

35 In this paper, it was found that venlafaxine could recover the quenched
36 fluorescence of acridine orange-alizarin red (AO-AR) system through charge-transfer
37 reaction between venlafaxine and alizarin red. Accordingly, a novel fluorescence
38 recovery method for the determination of venlafaxine hydrochloride was established.
39 Experimental results showed that this method was simple, fast, low cost and suitable
40 for the batch analysis of venlafaxine hydrochloride with good sensitivity and
41 selectivity, which had important significance for the quality control of pharmaceutical
42 production. To our knowledge, no paper using fluorescent recovery for the
43 determination of venlafaxine hydrochloride has been reported.

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44 ## 2 Experimental

45 ### 2.1 Apparatus

46 All fluorescence spectra were measured with a RF-5301 PC fluorescence
47 spectrophotometer (Shimadzu Corp., Japan). The UV-vis absorption spectra were
48 carried out on a UV-2102 PCS UV-vis spectrophotometer (Unico Shanghai
49 Instrument Co., Ltd., China). All pH measurements were conducted with Ray
50 magnetic pHS-3C pH meter (Shanghai Jingke Industrial Co., Ltd., China). The
51 temperature was controlled by HH-38 thermostat water bath (Zhengzhou Changcheng
52 Technology and Business Co., Ltd., China).

53 ### 2.2 Reagents

54 All reagents were used as received without further experimental purification.
55 Acridine orange of analytical grade was purchased from Blue Season Science and
56 Technology Development Co., Ltd. (Shanghai, China). Alizarin red of analytical pure
57 was obtained from XinZhong Chemical Plant (Shanghai, China). Venlafaxine
58 hydrochloride was standard compound with the purity of 99.9% from National
59 Institutions for Food and Drug Control (Beijing, China). Analytical pure sodium
60 hydroxide (NaOH) was purchased from Shanghai Chemical Reagent Co., Ltd.
61 (Shanghai, China). Hydrochloric acid (HCl) of analytical grade was from Xilong
62 Chemical Plant (Guangdong, China). All other reagents were of analytical grade. The
63 water for the experiment was deionized water.

64 ### 2.3 Preparation of stock solutions

65 Three stock solutions with the concentration of 1.00×10^{-2} mol·L^{-1} were prepared

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66 by dissolving 0.3700 g acridine orange, 0.3603 g alizarin red and 0.3139 g
67 venlafaxine hydrochloride in deionized water with constant volume to 100 mL,
68 respectively. They were diluted to 5.00×10^{-4} mol·L^{-1} with deionized water for the
69 experiment. 0.1 M KH_2PO_4-Na_2HPO_4 buffer solution with pH=6.8 was also
70 prepared.

71 **2.4 Experimental procedure**

72 1.50 mL 5.00×10^{-4} mol·L^{-1} alizarin red solution, 1.00 mL of KH_2PO_4-Na_2HPO_4
73 buffer solution (pH=6.8) and 1.70 mL 5.00×10^{-4} mol·L^{-1} acridine orange solution
74 were added into a 10 mL volumetric flask, mixed well and reacted under stationary
75 condition for 5 min. After that, a certain amount of venlafaxine hydrochloride solution
76 was added, then diluted to the mark with deionized water and mixed well. The
77 volumetric flask with the mixed solution was placed in a constant temperature water
78 bath for 40 min at 30 °C. The solution's fluorescence intensity denoted as F_1 was
79 measured with 495 nm for excitation wavelength and 530 nm for emission
80 wavelength. The fluorescence intensity of blank solution without the addition of
81 venlafaxine hydrochloride was denoted as F_0 and was measured under the same
82 conditions. The intensity difference between them was calculated using $\Delta F=F_1-F_0$.

83 **2.5 Method principle**

84 Under the conditions of pH from 6 to 7, alizarin red had a strong absorption peak
85 centered at 525 nm. Acridine orange, a strong fluorescent material with tricyclic
86 aromatic plane structure, has a maximum emission wavelength between 526 and 530
87 nm [28]. In the solution of pH=6–7, because the maximum absorption wavelength of

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88 alizarin red is relatively consistent with the maximum emission wavelength of
89 acridine orange, efficient energy transfer can occur between them, which results in the
90 fluorescence quenching of acridine orange. Alizarin red, an anthraquinone compound,
91 contains electron-withdrawing groups such as carbonyl and sulfonic acid with
92 electron-deficient molecular structure of planar conjugated large π bond [29]. Thus,
93 charge transfer can occur with electron donor. When venlafaxine hydrochloride was
94 added to the solution, hydrochloric acid was neutralized in this near neutral solution,
95 then venlafaxine containing nitrogen group which has lone electron pair could behave
96 as electron donor. Charge transfer occurred with alizarin red can inhibit energy
97 transfer between acridine orange and alizarine red, so that the quenched fluorescence
98 of acridine orange was released. The process of fluorescence quenching and recovery
99 was illustrated in Fig. 1. There was a good linear relationship between the
100 fluorescence recovery and the concentration of venlafaxine hydrochloride in the
101 solution. Accordingly, a new fluorescence recovery method for the indirect
102 determination of venlafaxine hydrochloride was established.

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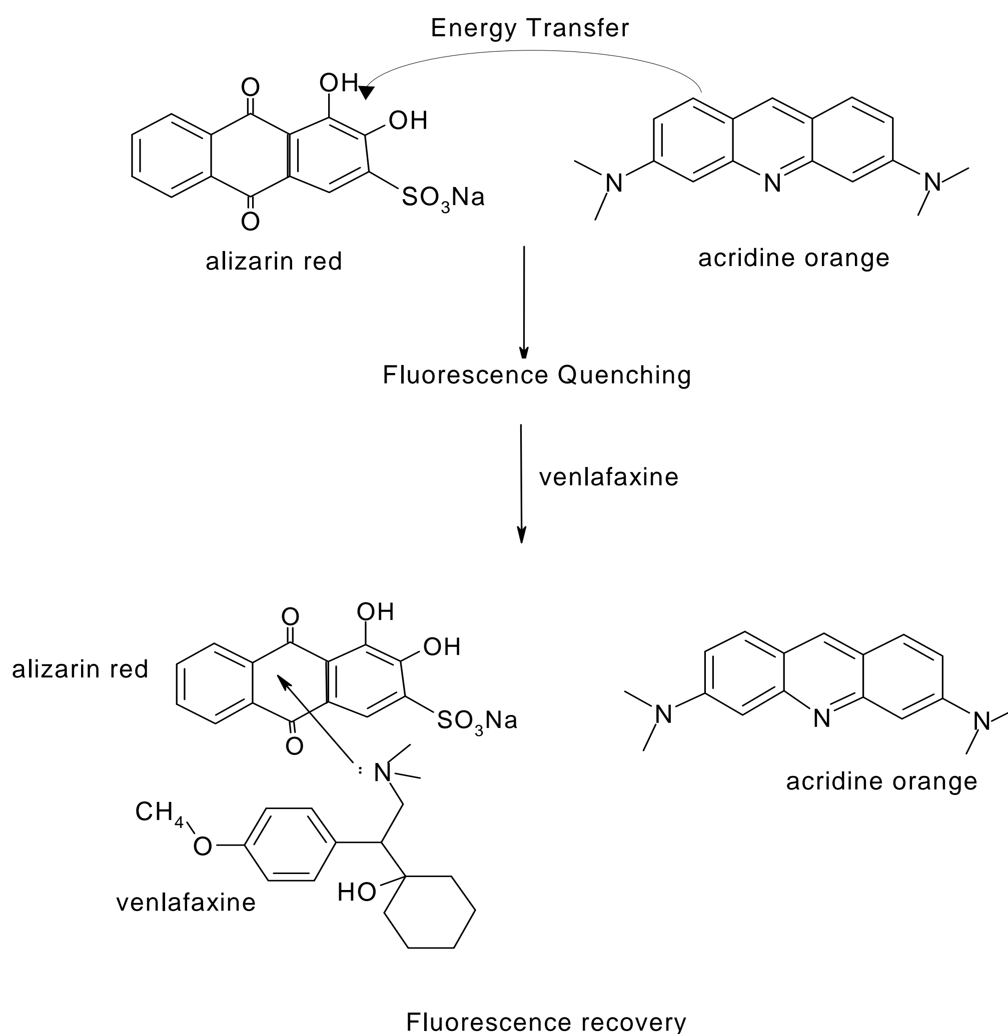

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104 Fig.1 The illustration for the method principle of fluorescence recovery in this system.

105 **3 Results and discussion**

106 **3.1 Fluorescence spectra of different solutions**

107 In accordance with the experimental method, fluorescence spectra of acridine
108 orange, acridine orange-alizarin red and acridine orange-alizarin red-venlafaxine
109 hydrochloride solutions containing 1.00 mL KH_2PO_4-Na_2HPO_4 buffer were obtained
110 and shown in Fig. 2a, b and c, respectively. It can be seen from Fig. 2a that acridine
111 orange has an intense emission peak with the maximum wavelength at 530 nm.
112 Observed from Fig. 2b and Fig. 2c, alizarin red quenches significantly the

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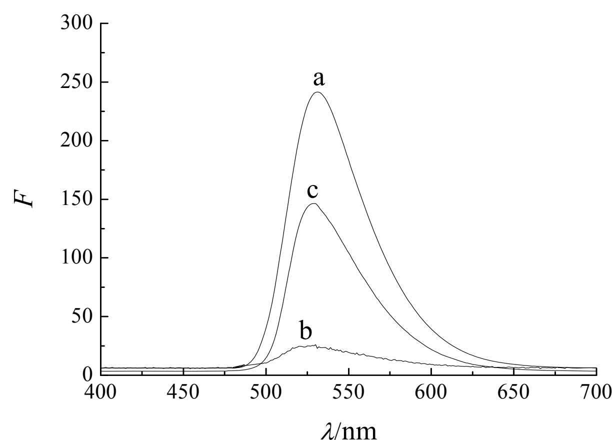
113 fluorescence of acridine orange and venlafaxine hydrochloride anti-quenches the

114 fluorescence of acridine orange-alizarin red solution.



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116 Fig. 2 Fluorescence spectra of different solutions: (a) 8.5×10^{-5} mol·L^{-1} AO; (b) a+7.5×10^{-5}

117 mol·L^{-1} AR; (c) b+6.0×10^{-5} mol·L^{-1} VLX.

118 In addition, fixed acridine orange concentration of 8.5×10^{-5} mol·L^{-1}, alizarin red

119 concentrations of 7.5×10^{-5} mol·L^{-1}, and added different amounts of venlafaxine

120 hydrochloride solution, the fluorescence spectra of these mixed solutions were

121 obtained and shown in Fig. 3. It is illustrated that the fluorescence intensity enhanced

122 with the increasing concentration of venlafaxine hydrochloride. There was

123 proportional relationship between the enhanced fluorescence and the concentration of

124 venlafaxine hydrochloride. Based on this relationship, venlafaxine hydrochloride can

125 be measured quantitatively.

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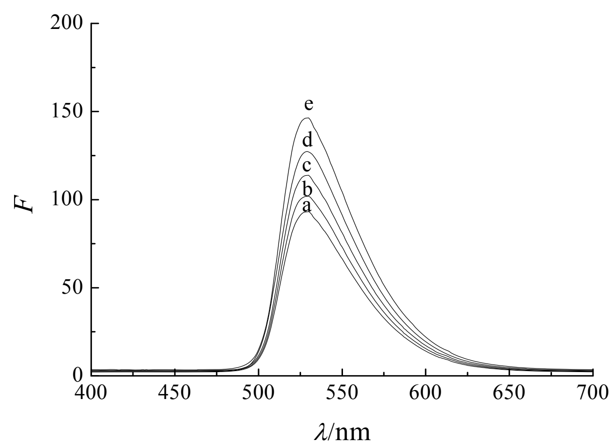

126

127 Fig. 3 Fluorescence spectra of VLX with different concentration in the solution of 7.5×10^{-5}

128 $mol\cdot L^{-1}$ AR and 8.5×10^{-5} $mol\cdot L^{-1}$ AO, in which the concentrations were 4.71, 9.42, 14.12, 18.83

129 and 23.54 $mg\cdot L^{-1}$ from curve a to curve e, respectively.

130 **3.2 Optimization of the determination conditions**

131 *3.2.1 Choice of the solution acidity and buffer system*

132 According to the experimental method, fixed other conditions, adjusted pH value of

133 solutions with 0.01 $mol\cdot L^{-1}$ HCl and NaOH, the effects of solution acidity on $\triangle F$ of

134 the reaction system was investigated and shown in Fig. 4. It can be seen that under

135 conditions of pH between 6 and 7, $\triangle F$ value reached maximum and became stable.

136 Because hydrochloric acid in venlafaxine hydrochloride was neutralized under near

137 neutral conditions, a stable charge transfer complex could be formed by reacting

138 between venlafaxine and alizarin red. Therefore, the pH of this experiment was

139 controlled between 6 and 7.

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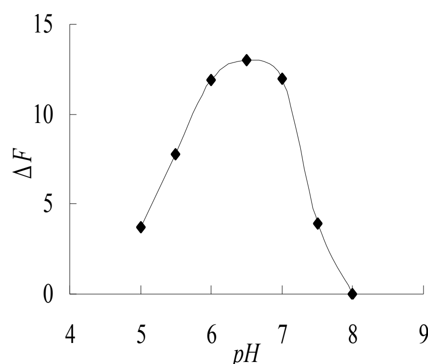

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141 Fig. 4 Influence of pH on △*F*, in which the solution contains 7.5×10^{-5} mol·L^{-1} AR , 8.5×10^{-5}

142 mol·L^{-1} AO and 15.7 mg·L^{-1} VLX

143 In this experiment, buffer solutions with pH of 6.8 including KH_2PO_4-Na_2HPO_4,

144 NaH_2PO_4-Na_2HPO_4, citric acid-Na_2HPO_4 and citric acid-sodium citrate were selected

145 to investigate the influence of buffer solution with different compositions on the

146 reaction system. The experimental results showed that when KH_2PO_4-Na_2HPO_4 buffer

147 solution was selected, △*F* value of the system reached maximum and stable. It may

148 be that citric acid and sodium citrate as organic molecules have unfavorable influence

149 on the organic reaction system. Thus, KH_2PO_4-Na_2HPO_4 was selected as buffer

150 solution in the experiment.

151 According to the experimental method, the amount of the buffer solution was also

152 investigated. It was found that when the amount of buffer solution was less than 0.8

153 mL, △*F* value enhanced with the increasing amount of buffer solution and when the

154 amount of buffer solution was in the range of 0.8–1.2 mL, △*F* value reached

155 maximum and substantially constant. In this study, the amount of buffer solution was

156 1.0 mL.

157 *3.2.2 The adding sequence of reagents*

158 The effect of adding sequence on the reaction system was also investigated. Five

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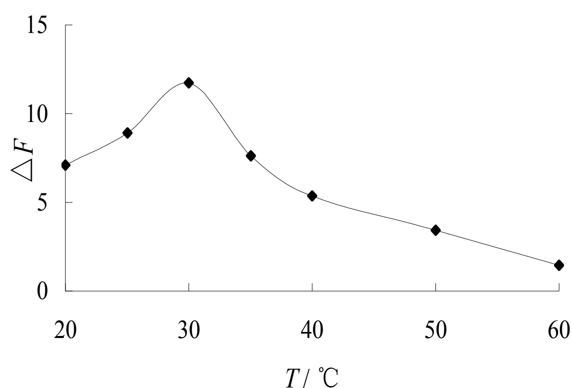
159 kinds of adding sequences were tested, i.e. VLX → buffer solution → AR → AO, AR
160 → buffer solution → VLX → AO, AR → buffer solution → AO → VLX, AO → AR
161 → buffer solution → VLX and AO → buffer solution → AR → VLX. Finally, it was
162 found that when the adding sequence was AR → buffer solution → AO → VLX, $\triangle F$
163 value of the system was maximum and stable. Thus, it was chosen as the optimal
164 adding sequence in this research.

165 *3.2.3 Choice of the reaction temperature*

166 Within the temperature range of 20–60 °C, the influence of reaction temperature on
167 $\triangle F$ value was investigated and shown in Fig. 5. Observed from Fig. 5, when
168 temperature was below 30 °C, $\triangle F$ value enhanced with the increase of temperature
169 and reached maximum at 30 °C; however, $\triangle F$ value decreased with the increasing
170 temperature when temperature was above 30 °C. We presume that at first the
171 increasing temperature is helpful to the formation of charge transfer complex and
172 energy transfer in this system, then the promoted dissociation of complex is
173 unfavorable for energy transfer with the continuous increase of temperature. Thus, 30
174 °C was chosen as optimum temperature for this experiment.



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176 Fig. 5 Influence of temperature on $\triangle F$, in which the solution contains 7.5×10^{-5} mol·L^{-1} AR ,

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177 8.5×10^{-5} mol·L^{-1} AO and 15.7 mg·L^{-1} VLX

178 *3.2.4 Choice of the reaction time*

179 With the temperature at 30 °C, the effect of reaction time from 10 to 60 min on △*F*

180 was illustrated in Fig. 6. As shown in Fig. 6, at first, △*F* value of the system was

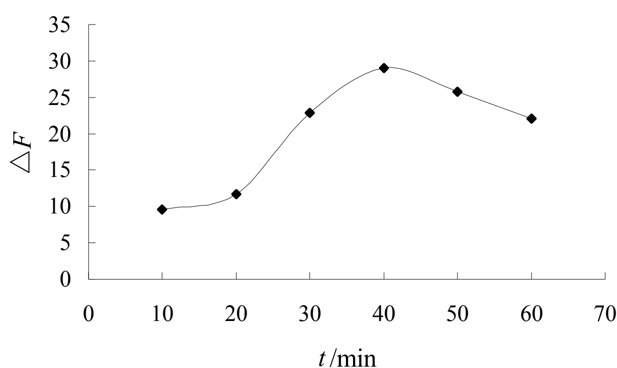
181 increasing with the increase of reaction time, and then △*F* value reached maximum

182 when the system reacted for 40 min, finally, △*F* value gradually decreased with the

183 reaction time more than 40 min. This result showed that too long reaction time could

184 cause the decomposition of charge transfer complex. In this paper, reaction time of 40

185 min was selected.



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187 Fig. 6 Influence of reaction time on △*F*, in which the solution contains 7.5×10^{-5} mol·L^{-1} AR ,

188 8.5×10^{-5} mol·L^{-1} AO and 20.0 mg·L^{-1} VLX

189 *3.2.5 Optimization of the amount of acridine orange*

190 In order to investigate the effect of the amount of acridine orange on △*F*, fixed other

191 conditions, changed the amount of acridine orange, a series of △*F* values were

192 measured according to the experimental method. The results shown in Fig. 7 indicated

193 that when the amount of acridine orange solution was within

194 8.0×10–5~9.0×10–5mol.L-1, △*F* value reached maximum and relatively stable, then

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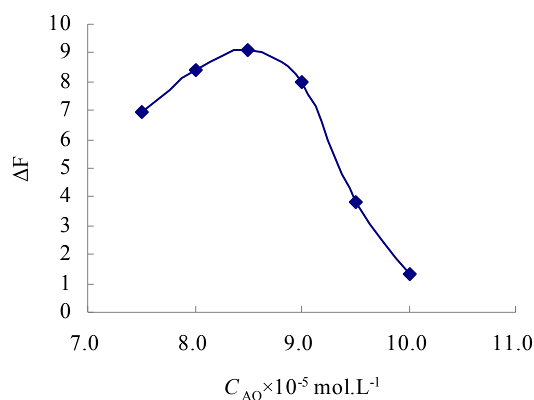
195 with the continuous increase of the concentration of acridine orange, ΔF value
196 decreased. Thus, the best proportion of alizarin red, acridine orange and venlafaxine
197 hydrochloride should be controlled at 1.5:1.7:≤ 1 in this reaction system.



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199 Fig. 7 Effect of the amount of AO on $\triangle F$, in which the different amount of solution was added into the
200 reaction solution containing 7.5×10^{-5} mol·L^{-1} AR and 15.7 mg·L^{-1} VLX.

201 **3.3 Calibration curve and detection limit of the method**

202 Under optimum conditions, fixed the amount of other solutions, changed the
203 amount of venlafaxine hydrochloride solution, a series of reaction solutions were
204 prepared. In accordance with the test method, $\triangle F$ values of the solutions were
205 measured. A standard curve was obtained by taking $\triangle F$ as the ordinate and mass
206 concentration (mg/L) of venlafaxine hydrochloride as the abscissa. The regression
207 equation was $\triangle F=0.4997m_{\mathrm{VLX}}+0.745$ with the correlation coefficient R of 0.9976.
208 The results indicated that there was a good linear relationship between $\triangle F$ value and
209 the concentration of venlafaxine hydrochloride in the range of 3.49–31.4 mg·L^{-1}. The
210 detection limit (3σ) obtained according to IUPAC regulation was 1.65 mg·L^{-1}.

211 **3.4 Precision and accuracy**

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212 To assess the precision and accuracy of the method, the determination of 20.0
213 $mg \cdot L^{-1}$ venlafaxine hydrochloride was carried out for eleven times. The average value
214 for these determinations was 20.1 $mg \cdot L^{-1}$ with the relative standard deviation of
215 1.16%, which indicated that this method had good accuracy and precision.

216 **3.5 Interferences of co-existing foreign substances**

217 In order to investigate the possibility of practical application in the determination of
218 pharmaceutical preparation, interferences from excipients which were often contained
219 in tablets and capsules, such as glycerol, lactose, starch and stearic acid, etc. were
220 tested under optimum conditions. The results showed that the largest allowable
221 amount of foreign substances was 500 times of $K^+$and Na^+, 400 times of glucose and
222 ethanol, 300 times of stearic acid, 200 times of lactose, 100 times of surfactants
223 OP-10,50 times of dextrin and 10 times of starch based on the impact on fluorescence
224 intensity was not more than ± 5%. However, the impact.of amino acids and proteins on the
225 fluorescence intensity measurements is greater.

226

227 **3.6 Investigation on reaction mechanism**

228 The UV-vis absorption spectra of alizarin red, venlafaxine hydrochloride and
229 alizarin red-venlafaxine hydrochloride in KH_2PO_4-Na_2HPO_4 buffer solution (pH=6.8)
230 were investigated and the results were shown in Fig. 8. It can be seen that
231 venlafaxine hydrochloride has no absorption in the visible region (curve b), alizarin
232 red has the maximum absorption peak at ca. 522 nm (curve a), the maximum
233 absorption peak of alizarin red-venlafaxine hydrochloride is around 550 nm (curve c).

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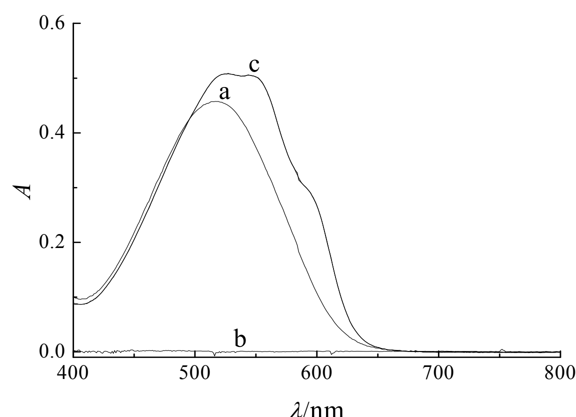
234 Compared with curve a, peak shape and height of curve c has significantly changed.

235 This indicated that the charge transfer reaction between alizarin red and venlafaxine

236 hydrochloride occurred to form a stable charge transfer complex under the conditions

237 of pH=6.8.



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239 Fig. 8 Absorption spectra of AR, VLX and AR+VLX in pH=6.8 buffer solution with the

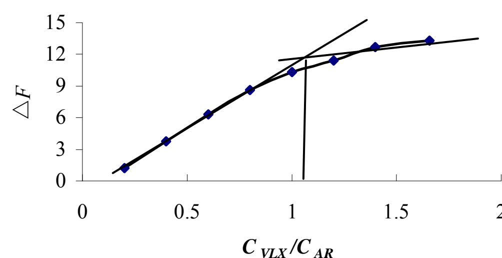
240 concentration of 7.5×10^{-5} mol·L^{-1} illustrated in curves a, b and c, respectively.

241 The mole ratio method was used to determine the composition ratio of charge

242 transfer complex. The results showed that the composition ratio of charge transfer

243 complex between venlafaxine hydrochloride and alizarin red was 1:1, as illustrated in

244 Fig. 9.



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246 Fig. 9 The stoichiometry of charge transfer complex determined by mole ratio method.

247 The necessary condition for the occurrence of energy transfer between substance

248 molecules is that the emission spectrum of energy donor has a certain degree of

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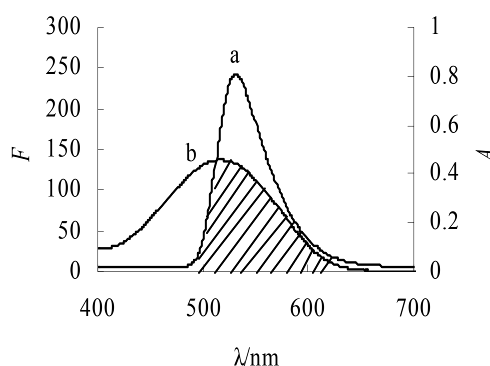
249 overlap with the absorption spectrum of energy receptor [30]. UV-vis spectrum of AR
250 and fluorescence emission spectrum of AO were shown in Fig. 10. The figure showed
251 that under conditions of KH_2PO_4-Na_2HPO_4 buffer solution (pH=6.8), fluorescence
252 emission peak of acridine orange centers around 530 nm, and maximum absorption
253 wavelength of alizarin red is at 522 nm. There exist 8 nm differences between the two
254 peaks and two spectra overlap partially. Thus, it is possible that energy transfer will
255 occur between acridine orange as energy donor and alizarin red as energy acceptor.



256

257 Fig. 10 (a) Fluorencence emission spectrum of AO (7.5×10^{-5} mol·L^{-1}); (b) absorption spectrum of
258 AR (7.5×10^{-5} mol·L^{-1}) in KH_2PO_4-Na_2HPO_4 buffer solution with pH=6.8.

259

260 In this paper, UV-vis absorption spectra of acridine orange, alizarin red, acridine
261 orange-alizarin red and acridine orange-alizarin red-venlafaxine hydrochloride were
262 also investigated. The results were shown in Fig. 11. It can be seen that acridine
263 orange has a maximum absorption at 440 nm and the maximum absorption peak of
264 alizarin red is at 522 nm, but when the two substances are mixed, the maximum
265 absorption peak moves to 470 nm. This indicated that the effective energy transfer
266 between acridine orange and alizarin red has occurred. When venlafaxine

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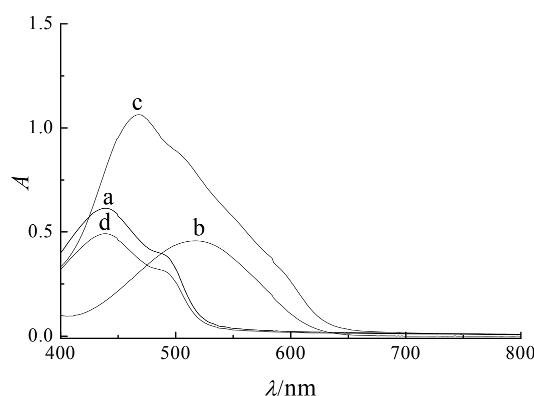
267 hydrochloride was added to this system, the absorption peak at 470 nm disappeared
268 and the characteristic absorption peak of acridine orange appeared again at 440 nm.
269 This showed that the energy transfer between acridine orange and alizarin red was
270 inhibited due to the charge transfer reaction of venlafaxine hydrochloride with alizarin
271 red.



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273 Fig. 11 Absorption spectra of different solutions: (a) AO (8.5×10^{-5} mol·L^{-1}); (b) AR (7.5×10^{-5}
274 mol·L^{-1}); (c) a+b; (d) c+VLX (6.0×10^{-5} mol·L^{-1}) in KH_2PO_4-Na_2HPO_4 buffer solution with
275 pH=6.8.

276 A series of exploring experimental results mentioned above proved that the reaction
277 principle described in section 2.5 is reasonable.

278 **3.7 Analytical application**

279 To investigate the possibility of practical application, the concentration of
280 venlafaxine hydrochloride in tablets and capsules was determined by this method. The
281 relative standard deviation was lower than 2.5%. The recovery of the method was
282 obtained through detecting three samples with adding different concentrations of
283 venlafaxine hydrochloride. The results were illustrated in Table 1 and the average
284 recovery was between 95.5% and 105%. HPLC [31] was also performed as

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285 comparative method. It was found that the result of determining venlafaxine
286 hydrochloride using this method was almost the same with HPLC. Thus, this method
287 was suitable to detect venlafaxine hydrochloride in tablets and capsules.
288
289
290 Table 1 Determination results of samples and recovery of the method (n=5).

Number	Labeled amount ($mg.L^{-1}$)	Found ($mg \cdot L^{-1}$)	RSD (%)	Added ($mg \cdot L^{-1}$)	Found ($mg \cdot L^{-1}$)	Average recovery (%)	HPLC ($mg \cdot L^{-1}$)
1	0.150	0.150	0.99	0.0314	0.180	95.5	0.149
				0.0627	0.212	98.9	
2	0.150	0.149	2.3	0.0314	0.182	105	0.148
				0.0627	0.212	100	
3	0.150	0.150	1.2	0.0314	0.181	98.7	0.151
				0.0627	0.212	98.9	

291 *Note: 1. Venlafaxine hydrochloride sustained-release tablets (manufacturers: Chengdu Nakasone*
292 *Pharmaceutical Group Co., Ltd., China; batch number: 100904; labeled amount: 75 mg/tablet).*
293 *2. Venlafaxine hydrochloride sustained-release tablets (manufacturers: Chengdu Nakasone*
294 *Pharmaceutical Group Co., Ltd., China; batch number: 100804; labeled amount: 75 mg/tablet).*
295 *3. Venlafaxine hydrochloride extended-release capsules (manufacturer: Wyeth Medica Ireland,*
296 *Ireland; batch number: 0901058; labeled amount: 75 mg/capsule).*

297 **4 Conclusions**

298 A novel and convenient technique for venlafaxine hydrochloride analysis has been
299 developed by fluorescence recovery on acridine orange-alizarin red system. Using this

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300 method, venlafaxine hydrochloride can be measured directly in aqueous solution and
301 the reagents required in this method are simple and easy to obtain. When this method
302 was used for the determination of venlafaxine hydrochloride in capsule and tablet
303 samples, the results were in excellent agreement with the labeled amount and that
304 measured by the HPLC [31] method. Comparing methods [1-19] mentioned in the
305 introduction, this method has the advantages of the simple sample pretreatment, measured at a
306 lower cost and no need to use the organic solvents. This method also has better sensitivity and
307 selectivity than methods [20-23] mentioned in the references [20-23].
308
309 **Acknowledgements**
310 This work was supported by the National Natural Science Foundation of China (No. 20942005).
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