Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

A Turn-On Fluorogenic Probe for MDMA from Ecstasy Tablets

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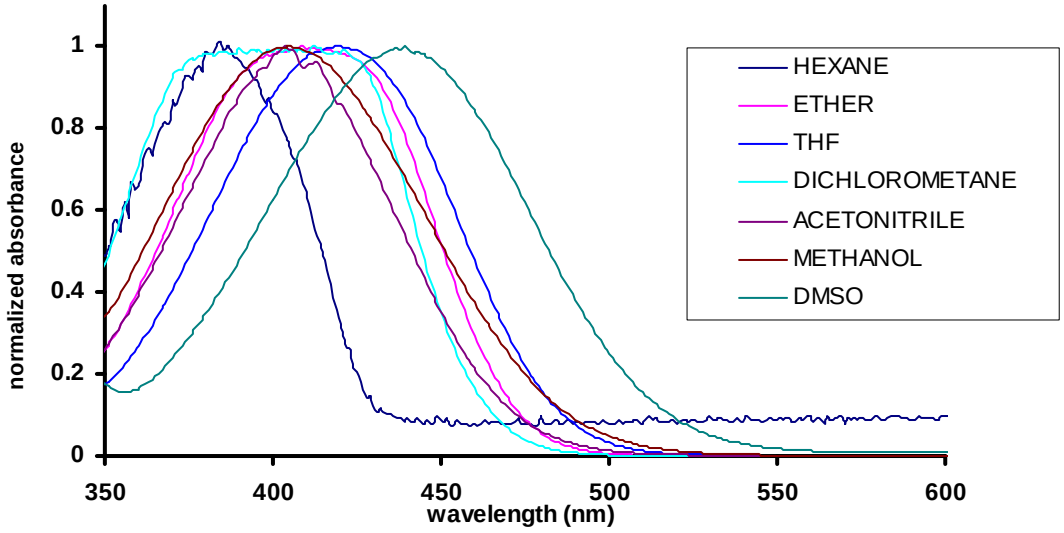
1.- Experimental Section:

Materials and methods: The reactions performed with air sensitive reagents were conducted under dry nitrogen. The solvents were previously distilled under nitrogen over calcium hydride or sodium filaments. Melting points were determined in a Gallenkamp apparatus and are not corrected. Infrared spectra were registered in a Nicolet Impact 410 spectrometer in potassium bromide tablets. NMR spectra were recorded in Varian Mercury-300 and Varian Unity Inova-400 machines, in DMSO-d_6, $CDCl_3$, CD_3CN, CD_3OD. Chemical shifts are reported in ppm with respect to residual solvent protons, coupling constants ($J_{X\text{-}X'}$) are reported in Hz. Elemental analyses of C, H and N were taken in a Leco CHNS-932. Mass spectra were taken in a Micromass AutoSpec machine, by electronic impact at 70 eV. Quantitative UV-visible measures were performed with a Varian, Cary 300 Bio UV spectrophotometer, in 1 cm UV cells at 25ºC. Fluorescence spectra were recorded in a Varian Cary Eclipse spectrofluorometer, in 1 cm quarz cells at 25ºC.

Solvatochromism of 1-dicyanomethylene-5-(4-aminophenyl)indane 1:

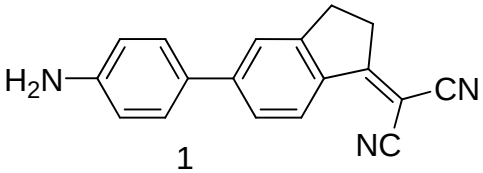
(1) (2) (3) (4) (5) (6) (7)

10^{-4} M solutions of 1 in (1) Hexane, (2) Et_2O, (3) THF, (4) CH_2Cl_2, (5) MeCN, (6) MeOH, (7) DMSO



Normalized absorbance spectra of 10^{-4} M solutions of 1 in Hexane, Et_2O, THF, CH_2Cl_2, MeCN, MeOH, DMSO

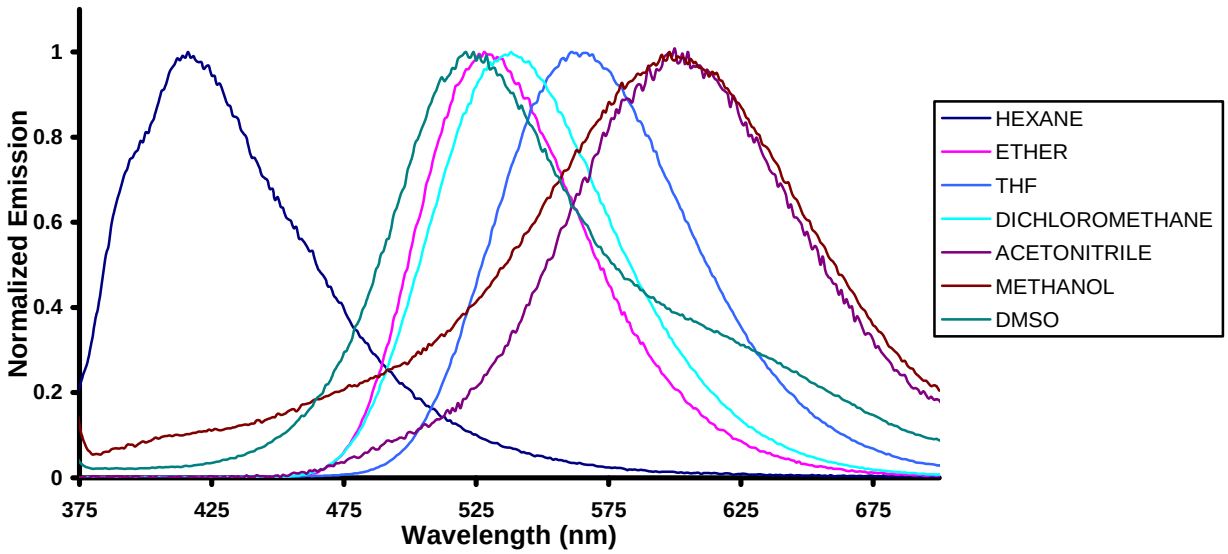
(1) (2) (3) (4) (5) (6) (7)



1

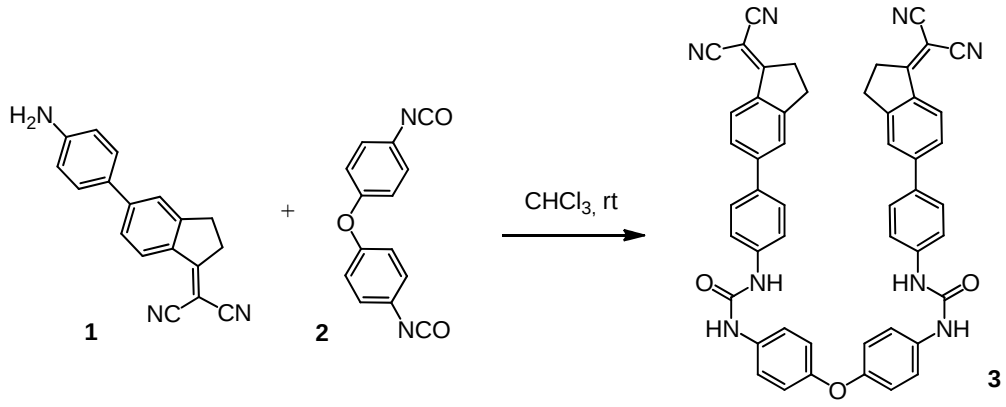
λ_{exc} = 366 nm

10^{-4} M solutions of 1 in (1) Hexane, (2) Et_2O, (3) THF, (4) CH_2Cl_2, (5) MeCN, (6) MeOH, (7) DMSO

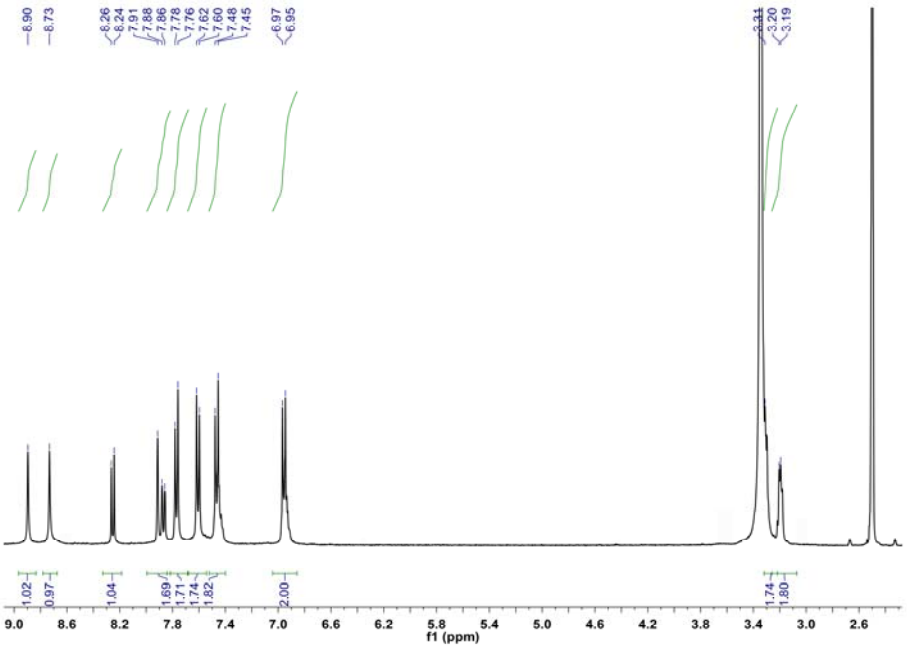


Normalized fluorescente spectra of 10^{-4} M solutions of 1 in Hexane, Et_2O, THF, CH_2Cl_2, MeCN, MeOH, DMSO; λ_{exc} = 366 nm.

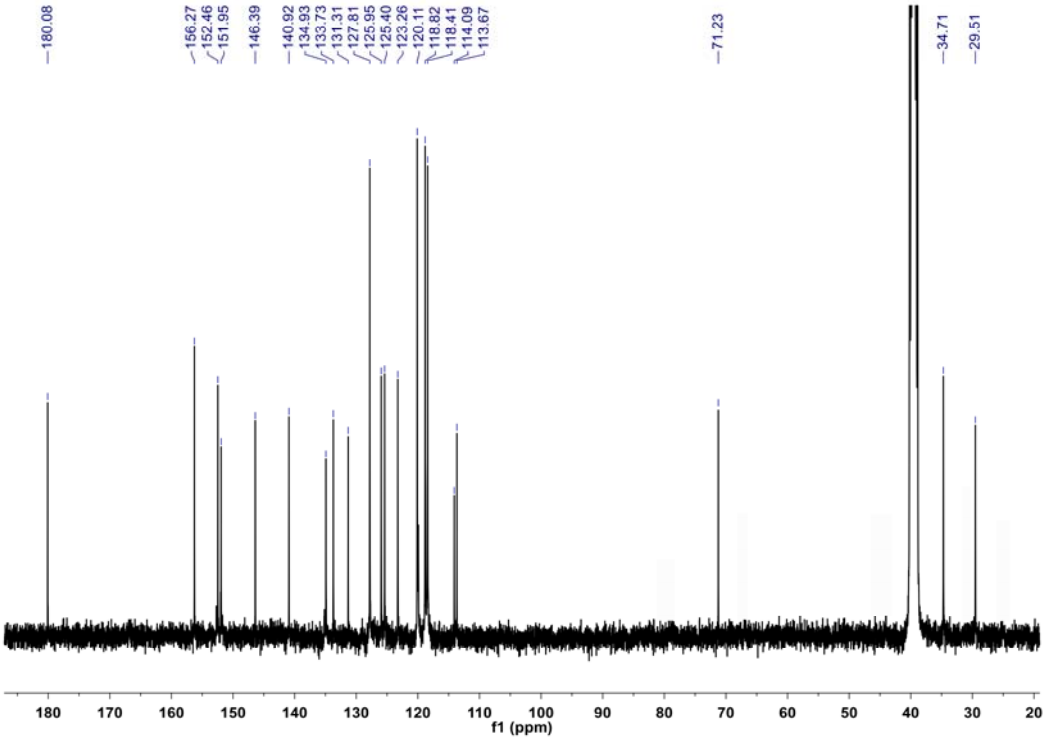
Synthesis of 4,4'-bis-{*N*-[4-(1-(dicyanomethyleneindan-5-yl)phenyl]-[*N*'-ureido]}diphenylether 3



1-Dicyanomethylene-5-(4-aminophenyl)indane **1** (300 mg, 1.10 mmol) was added to 4,4'-diisocyanatodiphenylether **2** (139 mg, 0.55 mmol) dissolved in $CHCl_3$ (20 mL) and the mixture was stirred at room temperature for 48 h. Cyclohexane (6 mL) was then added and the solid formed was filtered off, washed with $CHCl_3$ (2 portions of 4 mL) and dried. Compound **3** was obtained as a dark yellow solid (337 mg, 77%), mp >250 ºC. IR (KBr, cm^{-1}): 3316 (br, NH), 2221 (C≡N), 1654, 1598 and 1557 (C=O), 1510, 1239, 1184, 827. ^{1}H NMR (DMSO-d_6, 400 MHz): 8.90 (s, 2H, NH), 8.73 (s, 2H, NH), 8.25 (d, J = 8 Hz, 2H, ArH), 7.91 (s, 2H, ArH), 7.87 (d, J = 8 Hz, 2H, ArH), 7.77 (d, J = 8 Hz, 4H, ArH), 7.61 (d, J = 8 Hz, 4H, ArH), 7.46 (d, J = 8 Hz, 4H, ArH), 6.96 (d, J = 8 Hz, 4H, ArH), 3.30 (m, 4H, CH_2), 3.19 (m, 4H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz) 1780.08, 156.27, 152.46, 151.95, 146.39, 140.92, 134.93, 133.73, 131.31, 127.81 (CH), 125.95 (CH), 125.40 (CH), 123.26 (CH), 120.11 (CH), 118.82 (CH), 118.41 (CH), 114.09 (CN), 113.67 (CN), 71.23, 34.71 (CH_2), 29.51 (CH_2). MS (ESI) m/z (%): 795 (M + 1, 15). Anal. Calcd for $C_{50}H_{34}N_8O_3$: C, 75.55; H, 4.31; N, 14.10. Found: C, 75.39; H, 4.47; N, 13.92.



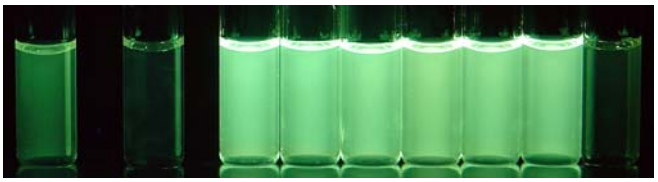
^{1}H NMR spectrum (DMSO-d_6, 400 MHz) of 4,4'-bis-{*N*-[4-(1-(dicyanomethyleneindan-5-yl)phenyl]-[*N*'-ureido]}diphenylether



^{13}C NMR spectrum (DMSO-d_6, 100 MHz) of 4,4'-bis-{*N*-[4-(1-(dicyanomethyleneindan-5-yl)phenyl]-[*N*'-ureido]}diphenylether

2.- Behaviour of 4,4'-bis-{*N*-[4-(1-(dicyanomethyleneindan-5-yl)phenyl]-[*N*'-ureido]}diphenylether 3 in the presence of common amines:

(a) Primary amines dissolved in water:



Ref. 3, (1) (2) (3) (4) (5) (6) (7) (8)

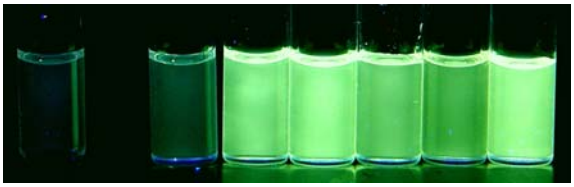
(1) Water (2) Propylamine, (3) Butylamine, (4) Cyclohexylamine, (5) Tert-butylamine, (6) Allylamine, (7) Benzylamine (8) Aniline.

Ref. 3, (1) (2) (3) (4)

(1) Propylamine, (2) Butylamine, (3) Pentylamine, (4) Heptylamine

Fluorescence of 10^{-4} M Solutions of **3** in DMSO +1 equivalent of each primary amine dissolved in water. λ_{exc} = 366 nm.

(b) Secondary amines dissolved in water:

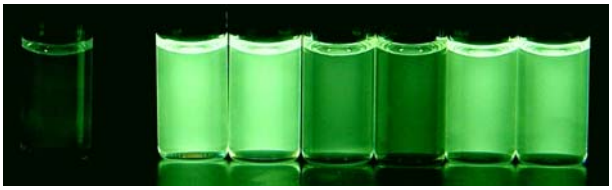


Ref. 3, (1) (2) (3) (4) (5) (6)

(1) Diphenylamine, (2) Diisopropylamine, (3) Diethylamine, (4) Diallylamine, (5) Diisobutylamine, (6) *N*-Methylbenzylamine

Fluorescence of 10^{-4} M Solutions of **3** in DMSO +1 equivalent of each secondary amine dissolved in water. λ_{exc} = 366 nm.

(c) Primary, secondary and tertiary amines dissolved in water:



Ref. 3, (1) (2) (3) (4) (5) (6)

(1) Pyrrolidine, (2) Piperidine, (3) Triethylamine, (4) Triisobutylamine, (5) Propylamine, (6) Butylamine

Fluorescence of 10^{-4} M Solutions of **3** in DMSO +1 equivalent of each primary, secondary or tertiary amine dissolved in water. λ_{exc} = 366 nm.

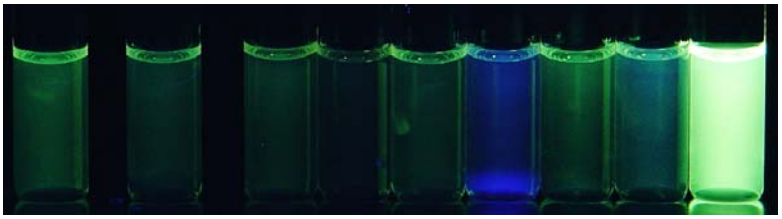
(d) Secondary and heterocyclic amines dissolved in water:

Ref. 3, (1) (2) (3)

(1) Pyrrolidine, (2) Pyridine, (3) Piperidine

Fluorescence of 10^{-4} M Solutions of **3** in DMSO +1 equivalent of each amine dissolved in water. λ_{exc} = 366 nm.

(e) Aromatic amines dissolved in water (except indication): Aniline (in DMSO), *o*-phenylenediamine, *m*-phenylenediamine, *p*-phenylenediamine, proton sponge, 9,10-diaminophenanthrene, pyrrolidine (added for comparison of its generated fluorescence to the effect of the rest of amines)

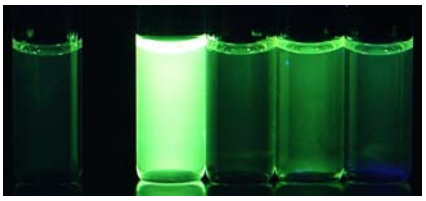


Ref. 3, (1) (2) (3) (4) (5) (6) (7) (8)

(1) Water, (2) Aniline, (3) *o*-Phenylenediamine, (4) *m*-Phenylenediamine, (5) *p*-Phenylenediamine, (6) Proton Sponge, (7) 9,10-Diaminophenanthrene, (8) Pyrrolidine

Fluorescence of 10^{-4} M Solutions of **3** in DMSO +1 equivalent of each amine dissolved in water. λ_{exc} = 366 nm.

(f) Heterocyclic amines dissolved in the corresponding solvent: Pyrrolidine (in water), pyrrole (in DMSO), indole (in water), carbazole (in DMSO-H_2O 80:20)



Ref. 3, (1) (2) (3) (4)

(1) Pyrrolidine, (2) Pyrrole, (3) Indole, (4) Carbazole

Fluorescence of 10^{-4} M Solutions of **3** in DMSO +1 equivalent of each amine dissolved in water. λ_{exc} = 366 nm.

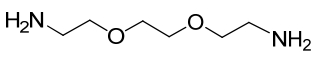
(g) Amines of diverse structure: Piperidine (in water), DBU (in water), DBN (in water), DABCO (in MeCN), triphenylamine (in MeCN), Hünig's base (in H_2O:DMSO 9:1), DMAP (in water), pyrrolidine (in water).

Ref. 3, (1) (2) (3) (4) (5) (6) (7) (8) (9)

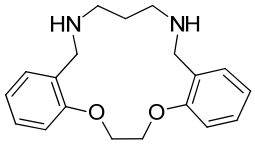
(1) Water, (2) Piperidine, (3) DBU, (4) DBN, (5), DABCO, (6) Triphenylamine, (7) Hünig's base, (8) DMAP, (9) Pyrrolidine

Fluorescence of 10^{-4} M Solutions of **3** in DMSO +1 equivalent of each amine dissolved in water. λ_{exc} = 366 nm.

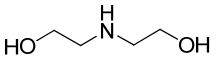
2,2'-(ethylenedioxy)bis(ethylamine)



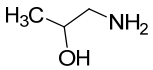
5,6,14,15-dibenzo-1,4-dioxa-8,12-diazacyclopentadeca-5,14-diene



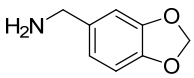
Diethanolamine



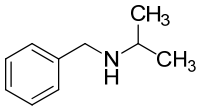
1-Amino-2-propanol



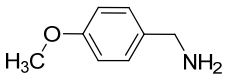
Piperonylamine



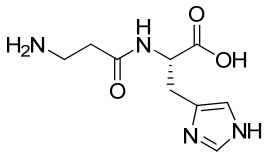
Benzylisopropylamine



4-methoxybenzylamine

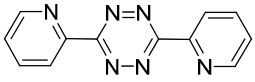


L-carnosine

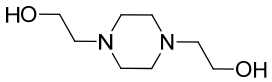


Structure of other amines that generate fluorescence in the presence of probe **3**.

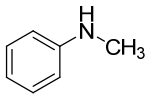
3,6-Di-(2-pyridyl)-1,2,4,5-tetrazine



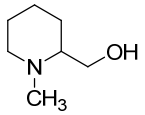
1,4-Bis(2-hydroxyethyl)piperazine



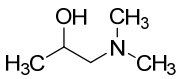
N-Methylaniline



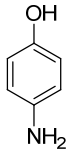
1-Methyl-2-piperidinemethanol



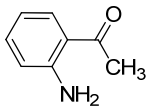
1-Dimethylamino-2-propanol



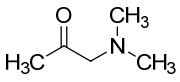
4-aminophenol



2'-Aminoacetophenone

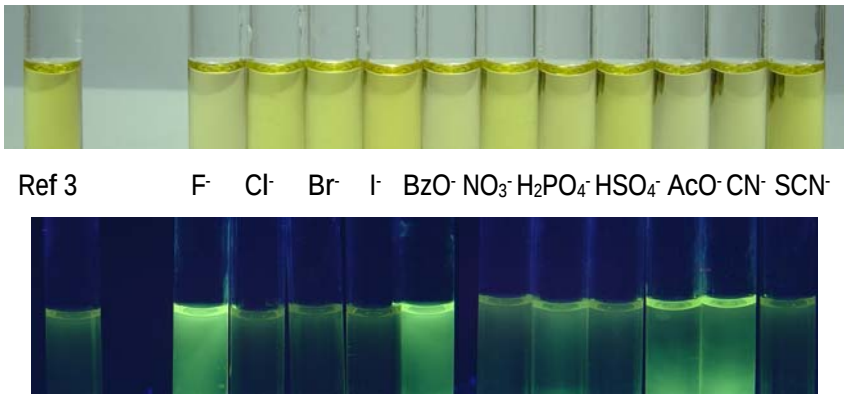


(Dimethylamino)acetone

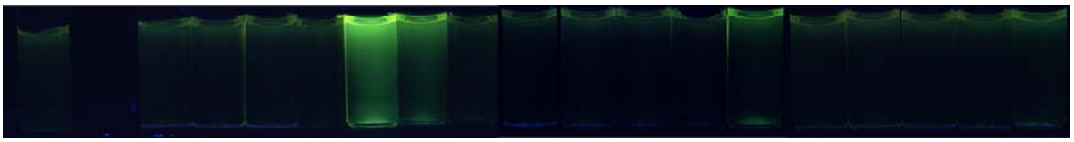


Structure of other amines that do not generate fluorescence in the presence of probe **3**.

3.- Behaviour of 3 in the presence of common anions and α-aminoacids

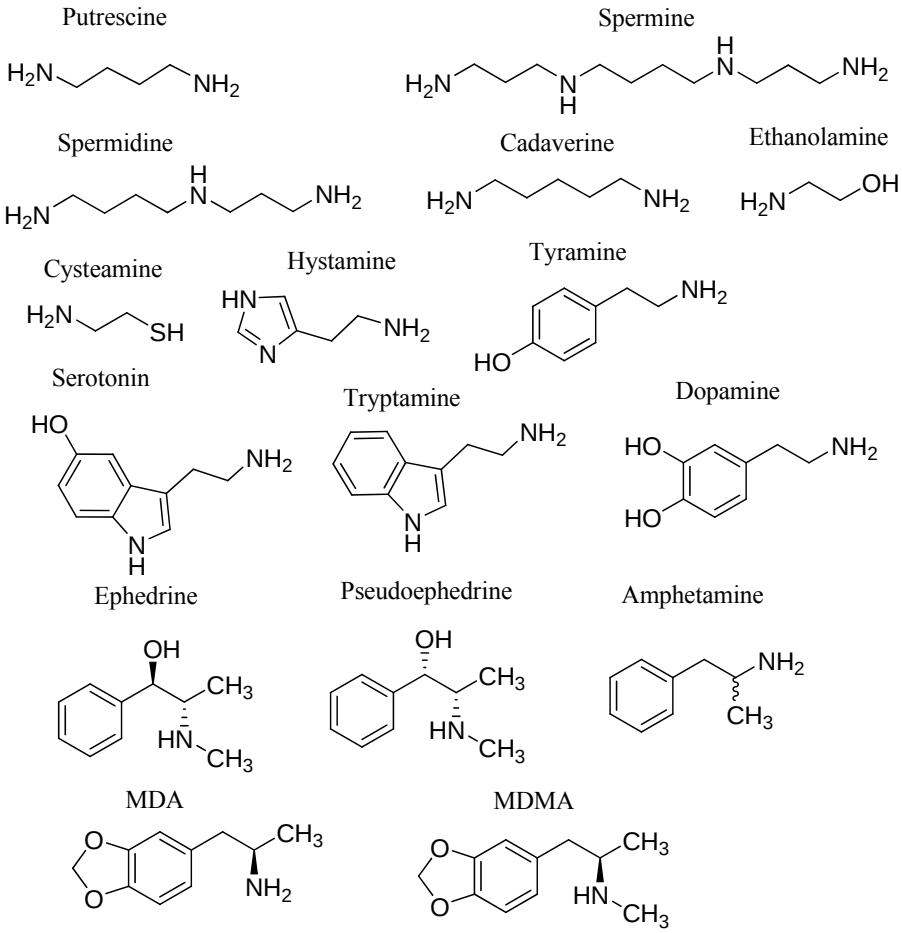


10^{-4} M Solutions of **3** in DMSO +1 equivalent of anion: F^-, Cl^-, Br^-, I^-, BzO^-, NO_3^-, $H_2PO_4^-$, HSO_4^-, AcO^-, CN^-, SCN^-. Up: White light. Down: λ_{exc} = 366 nm

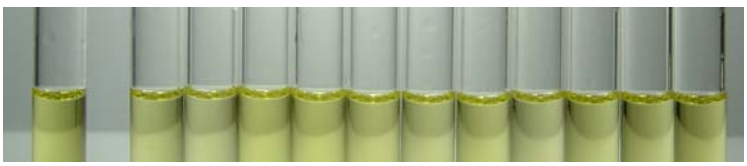


Fluorescence of 10^{-4} M Solutions of **3** in DMSO +1 equivalent of each aminoacid dissolved in water. Λ_{exc} = 366 nm

4.- Behaviour of 3 in the presence of biogenic amines:



Structure of biogenic amines and their mimics used in this study.



3 put spr ser dop cyt cad his spd eth try tyr

3 put spr ser dop cyt cad his spd eth try tyr

Effect of the addition of 2 equiv of biogenic amines in water to 10^{-4} M solutions of probe **3** in DMSO: **put** (putrescine), **spr** (spermine), **ser** (serotonin), **dop** (dopamine), **cyt** (cysteamine), **cad** (cadaverine), **his** (□istamine), **spd** (spermidine), **eth** (ethanolamine), **try** (tryptamine), **tyr** (tyramine). Up: White light. Down: λ_{exc} = 366 nm

3 eph, peh, anf, MDA, MDMA.

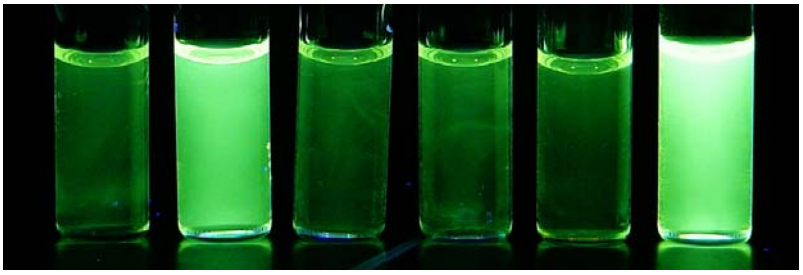
3 eph pse amp MDA MDMA

Effect of the addition of 2 equivalents of ephedrines or amphetamines in water to 10^{-4} M solutions of **3** in DMSO: **eph** (ephedrine), **pse** (pseudoephedrine), **amp** (±amphetamine), **MDA** (±3,4-methylenedioxyamphetamine), **MDMA** (±3,4-methylenedioxymethamphetamine).

5.-Detection of MDMA in the presence of some interfering additives as can be found in a tablet of ecstasy:

MDMA (3,4-methylenedioxymethamphetamine) also known as ecstasy is a recreational drug with psychoactive properties that acts directly on the action of neurotransmitters causing alterations of the mood. Usually the drug is consumed in the form of tablets, but never in a pure state because in the composition of the final dose some excipients are added to reduce the final price. The adulterants are normally gypsum or chalk, caffeine, sucrose, mannitol or aspirin, and the percentage in the sample may vary from 30% in the ecstasy tablets to 80% in the so-called crystal. In this experiment the composition of an ecstasy tablet has been simulated and we have studied the signal generated by **3** (10^{-4} M solution in DMSO) in the presence of every component and the mixture.

Qualitative study: A 10^{-4} M solution of **3** in DMSO was tested in the presence of solutions of every component and the mixture.



3 MDMA sucrose chalk caffeine ecstasy

3: 10^{-4} M solution of **3** in DMSO

MDMA: **3** + 1 equiv MDMA

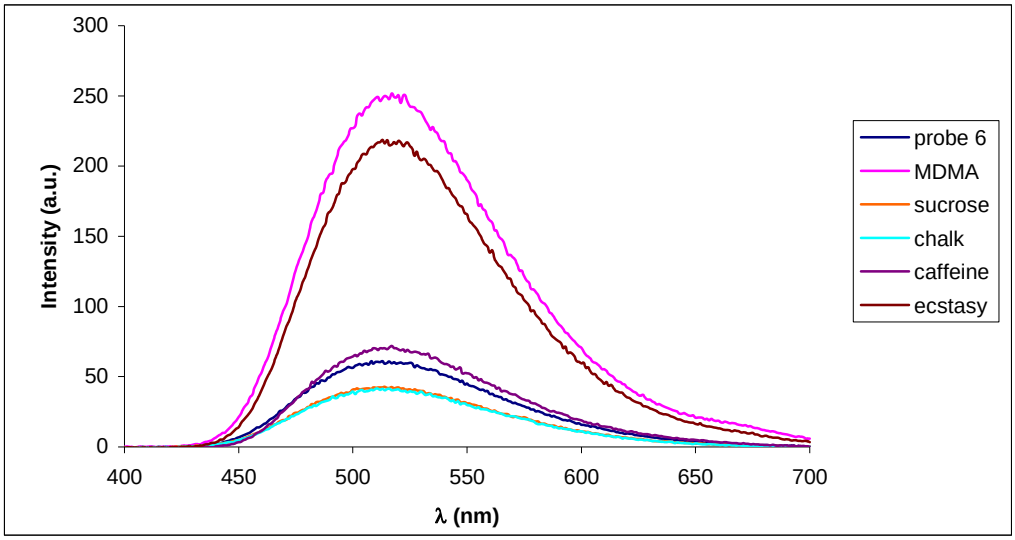
sucrose: **3** + 1 equiv sucrose in water

chalk: **3** + filtered chalk dispersion in water

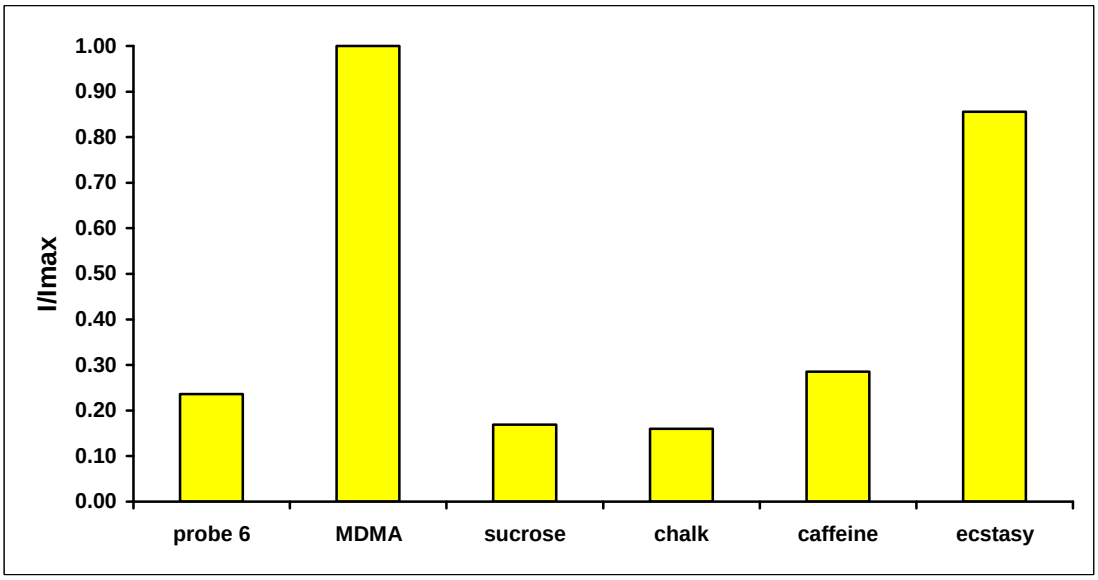
caffeine: **3** + 1 equiv caffeine in water

ecstasy: **3** + 20 µl of a solution of MDMA (45%) + sucrose + chalk + caffeine in water

Fluorimetric study: The previous solutions were measured in fluorescence emission (λ_{exc} = 288 nm).



Fluorescence measurements of the samples, λ_{exc} = 288 nm

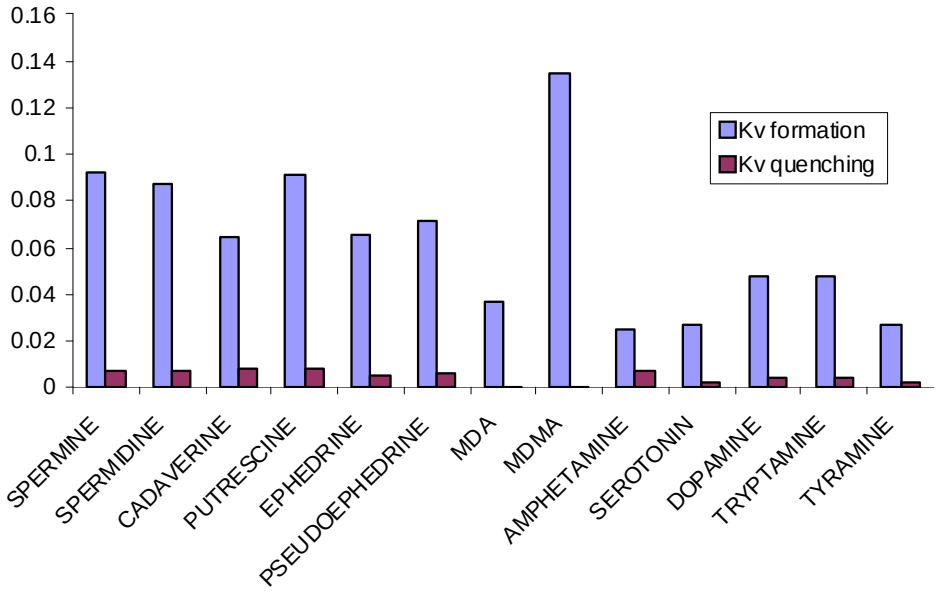


Normalized intensity of the fluorescence maximum in each case, λ_{max} = 517 nm

6.-Kinetic supporting information:

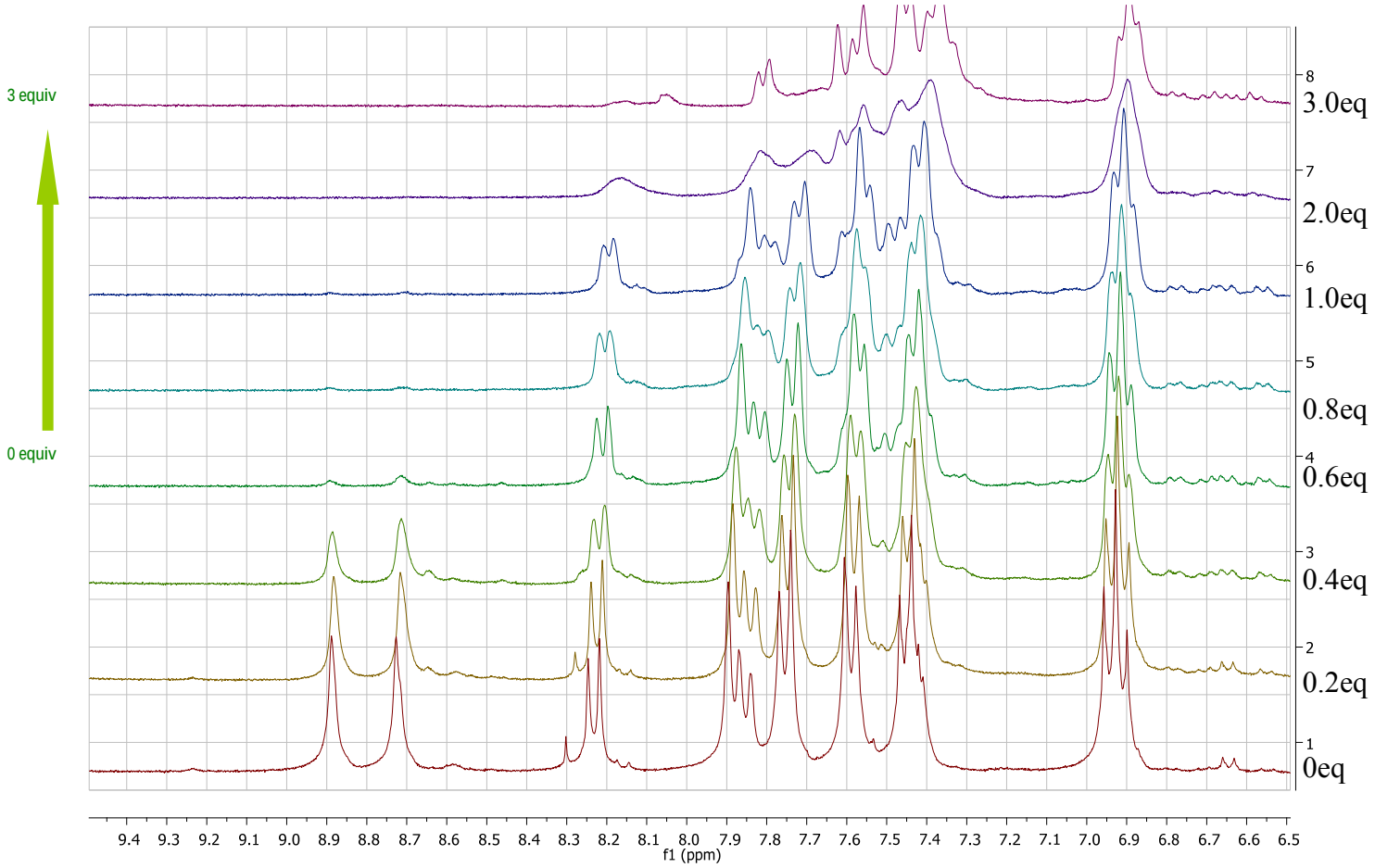
Table 1. Rate constants, s^{-1}, for the formation, k_v^f, and quenching, k_v^q, of the fluorescence complexes generated with probe **3** and different amines. C_{amine}= 8.3×10^{-4} M in water, C_6 = 8.3×10^{-5} M in DMSO; in the mixture 16 % v/v water/DMSO, T = 25ºC.

	tyramine	tryptamine	putrescine	cadaverine
$10^2\ k_v^f$	2.65±0.01	4.78±0.01	9.08±0.01	6.43±0.01
$10^3\ k_v^q$	2.2±0.6	4.4±0.3	8.1±0.4	8±1
	spermine	spermidine	dopamine	serotonin
$10^2\ k_v^f$	8.72±0.01	9.24±0.01	4.78±0.01	2.65±0.01
$10^3\ k_v^q$	7.5±0.5	7.2±0.6	4.4±0.5	2.2±0.2
	ephedrine	pseudoephedrine		amphetamine
$10^2\ k_v^f$	6.54±0.01	7.16±0.01		2.44±0.01
$10^3\ k_v^q$	5.4±0.3	6±1		7.2±0.1
	MDA		MDMA	
$10^2\ k_v^f$	3.66±0.02		13.5±0.3	
$10^4\ k_v^q$	5±1		44.2±0.2	

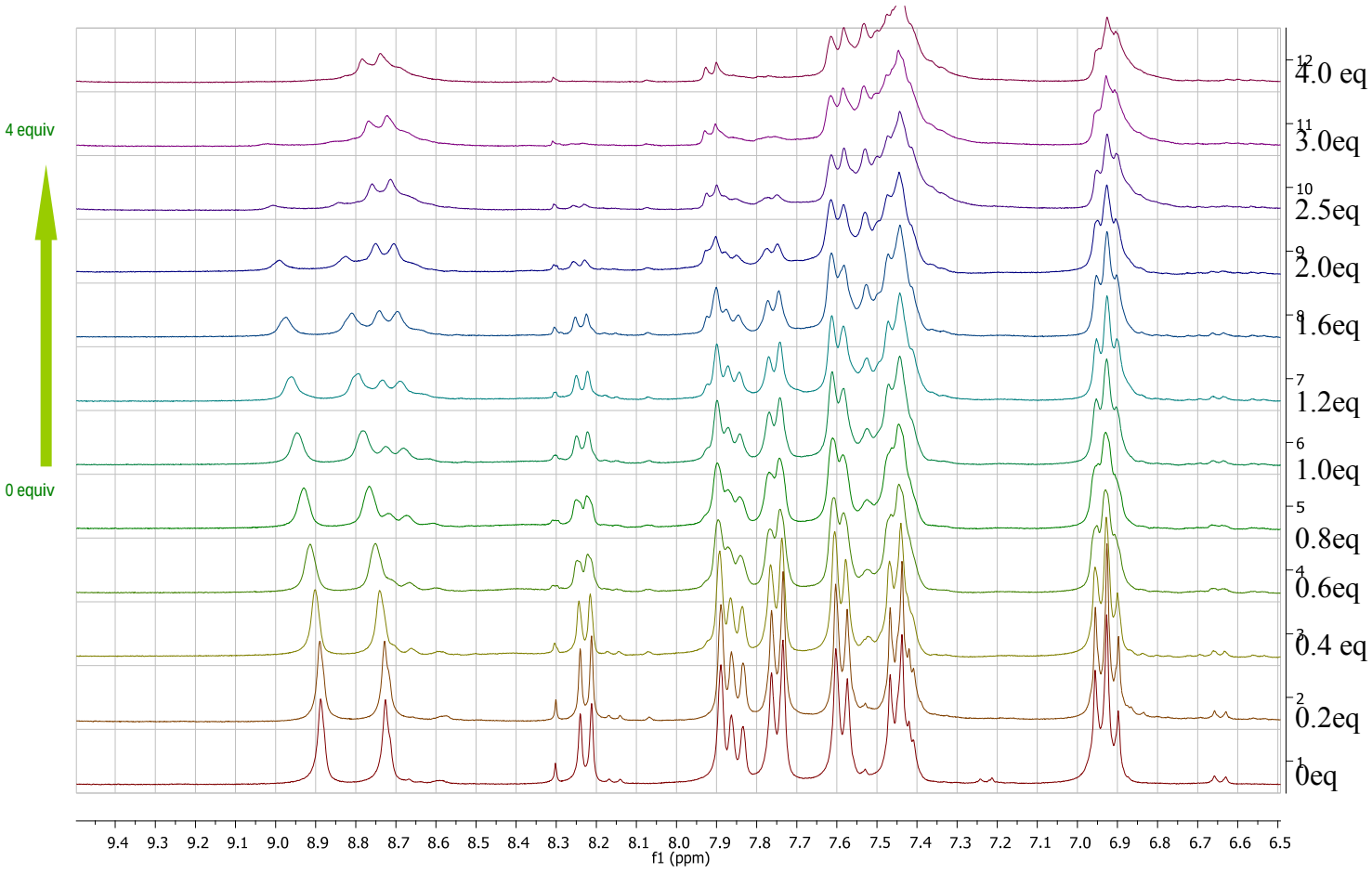


7.- ^{1}H NMR TITRATION STUDIES

^{1}H NMR titration of 3 (10^{-1} M solution in DMSO-d_6) with pyrrolidine in D_2O.



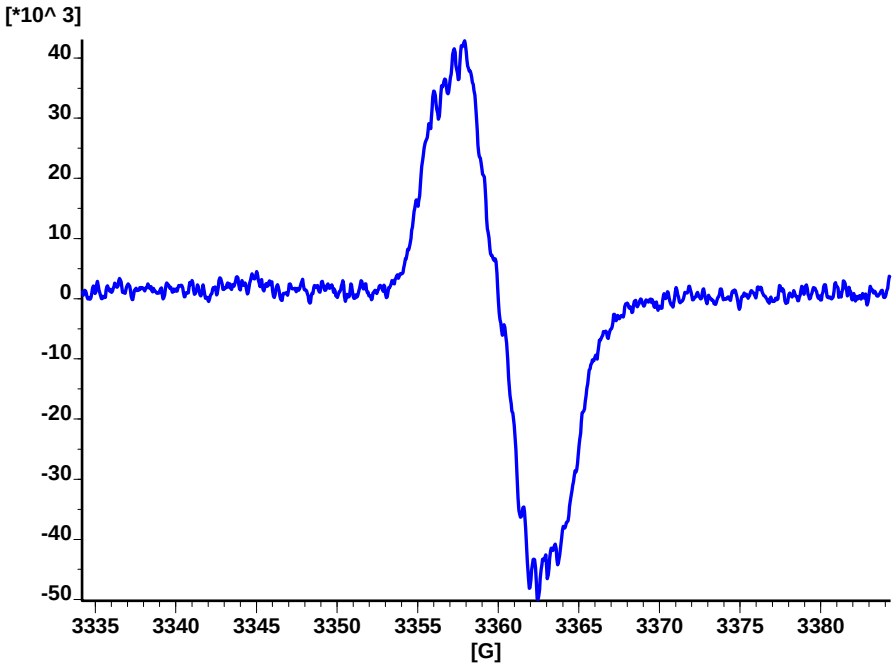
^{1}H NMR titration of 3 (10^{-1} M solution in DMSO-d_6) with pyrrolidine in DMSO-d_6.



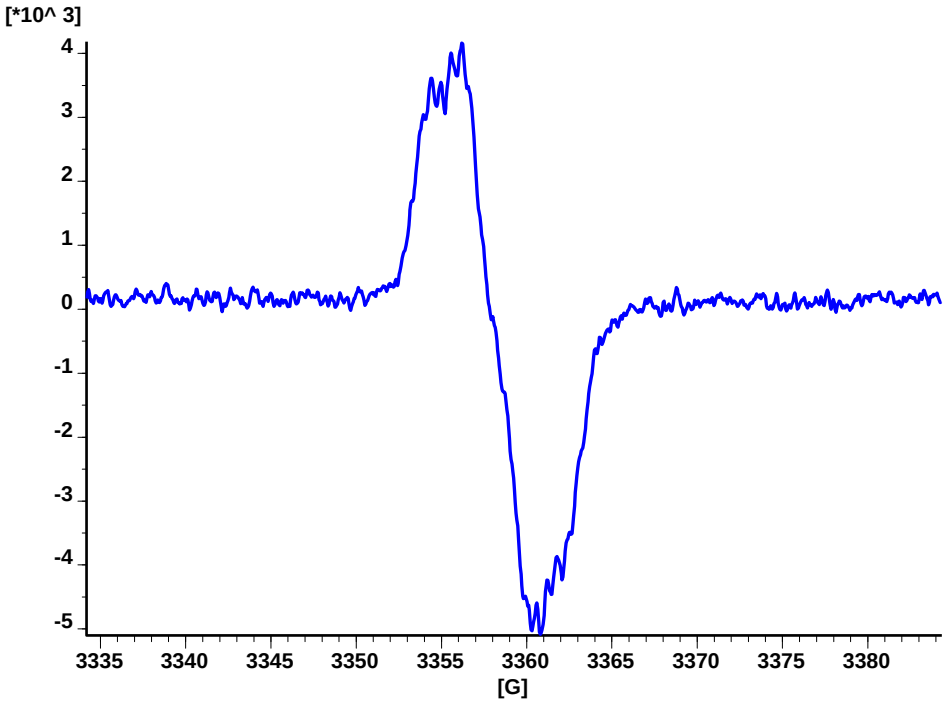
Titration of **3** and pyrrolidine showed complexation and proton-deuterium interchange when the analyte was dissolved in D_2O, and complexation with desymmetrization of the signals with appearance of different proton signals belonging to the diversely complexated urea groups. In the presence of excess of pyrrolidine the aromatic signals enlarged with an extensive loss of resolution. This effect could be due to dilution but also could be due to formation of bi-radical species. Therefore we performed several EPR experiments. First the EPR of the mixture from the ^{1}H NMR titration of **3** (10^{-1} M solution in DMSO-d_6) with pyrrolidine in DMSO-d_6 was kept into a capillary glass tube, then put into an EPR tube and then subjected to room temperature EPR acquisition in an EPR spectrometer BRUKER EMX10/12 operating in Band X (9-10 GHz) with calibration by DPPH. The EPR spectrum showed a large multiplet with low resolution, which was similar when taken from the NMR titration or from a mixture of the probe **3** and 1 equivalent of pyrrolidine in DMSO. The EPR spectrum did not change on time within minutes. The solvent itself did not show any appreciable EPR signal.

8.- EPR Studies:

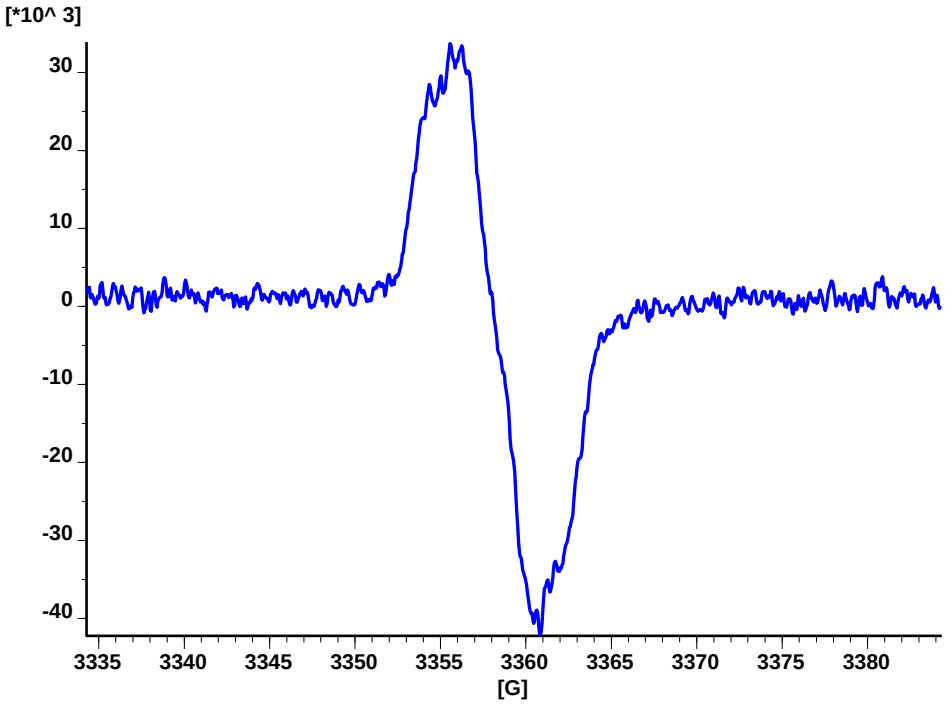
EPR of a mixture of 3 (10^{-1} M solution in DMSO-d_6) and 4 equivalents of pyrrolidine in DMSO-d_6 after the NMR titration.



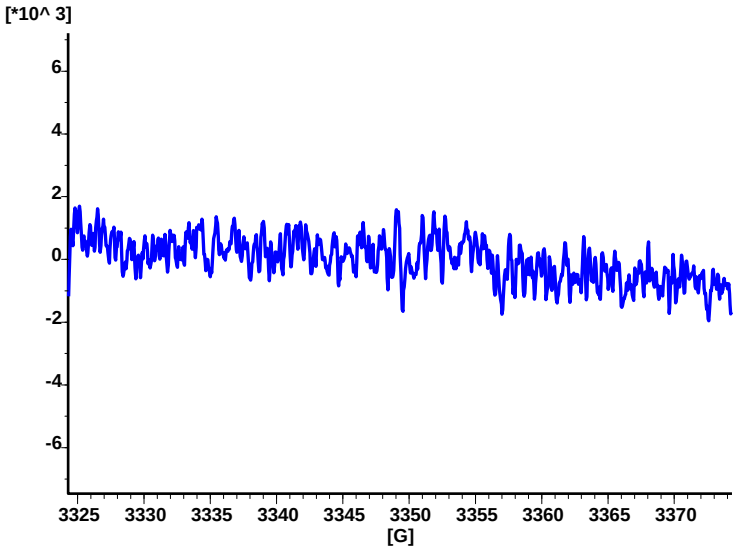
EPR of a mixture of 3 (10^{-1} M solution in DMSO) with 1 equivalent of pyrrolidine in DMSO, 15 minutes after preparation.



EPR of a mixture of 3 (10^{-1} M solution in DMSO) with 1 equivalent of pyrrolidine in DMSO, recorded 15 minutes later than the previous one.



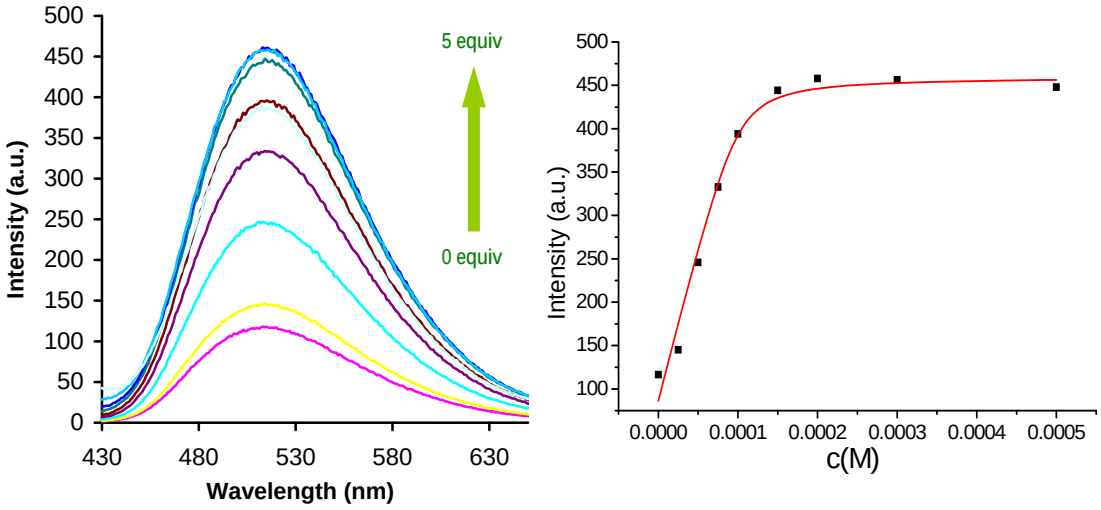
EPR of the background solvent, DMSO.



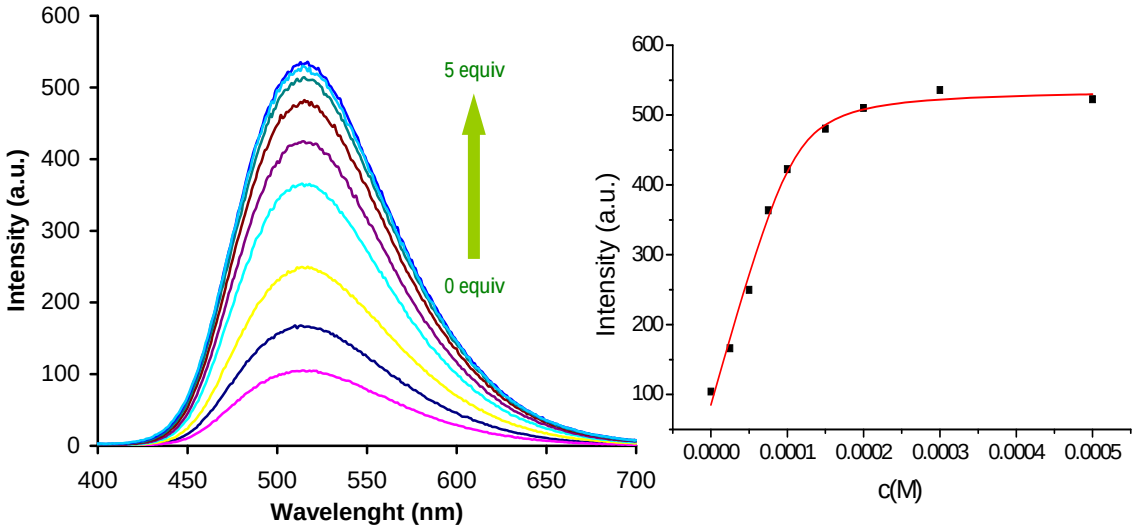
9.- Fluorimetric titrations of 3 in DMSO with amines in water:

Table 2. Binding equilibrium constants for 1:1 complexes obtained from fluorescence titrations of 10^{-4} M solutions of **3** in DMSO and amines in water.

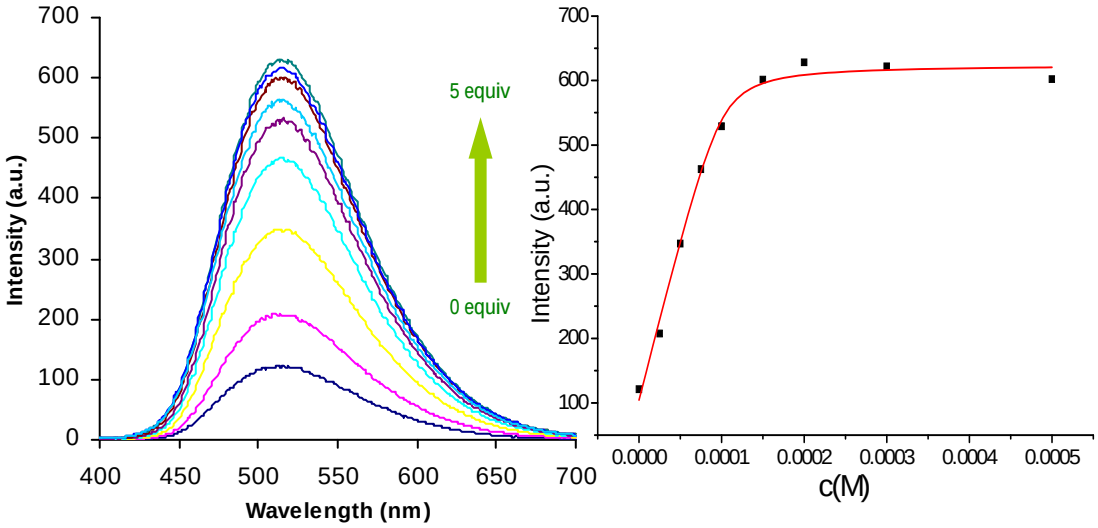
	tyramine	tryptamine	putrescine	cadaverine
Log K_{eq}	5.17 ± 0.36	5.40 ± 0.09	5.49± 0.17	6.23 ± 0.50
	spermidine	spermine	histamine	dopamine
Log K_{eq}	5.48 ± 0.25	5.78 ± 0.24	4.43± 0.14	4.09 ± 0.12
	serotonin	cysteamine	ethanolamine	
Log K_{eq}	4.83 ± 0.19	3.53 ± 0.14	4.41 ± 0.18	
	cadaverine HEPES pH 8.2		dopamine HEPES pH 8.2	
Log K_{eq}	4.31 ± 0.53		5.14 ± 0.23	
	ephedrine	pseudoephedrine	amphetamine	MDA
Log K_{eq}	4.50±0.11	4.36 ± 0.08	5.35± 0.17	4.29±0.13
	MDMA	MDA HEPES pH 8.2	MDMA HEPES pH 8.2	
Log K_{eq}	4.12±0.12	3.95 ± 0.10	4.23 ± 0.09	



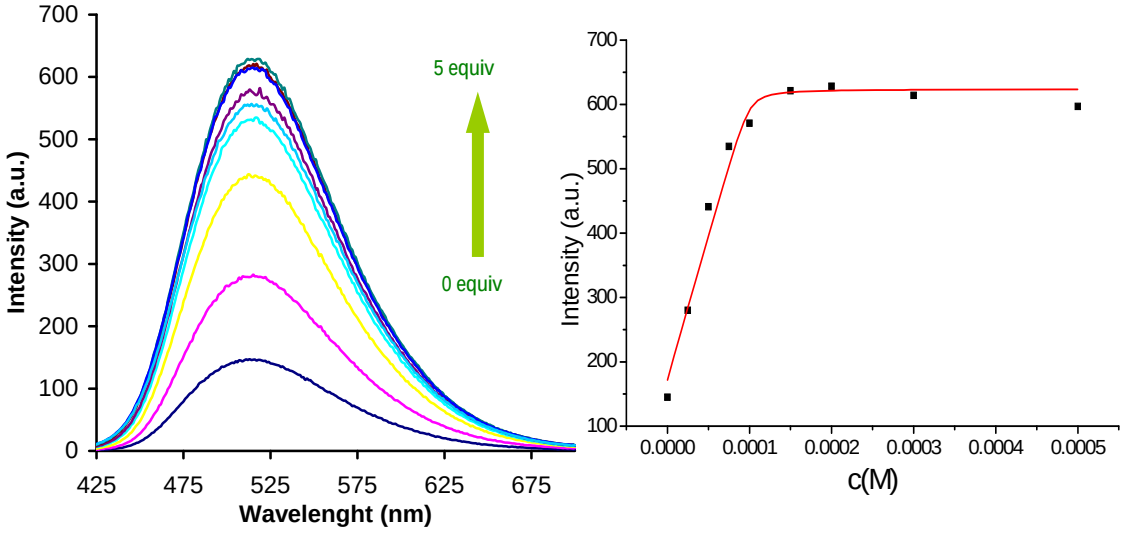
Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with tryptamine in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 517 nm) for the titration of **3** with tryptamine.



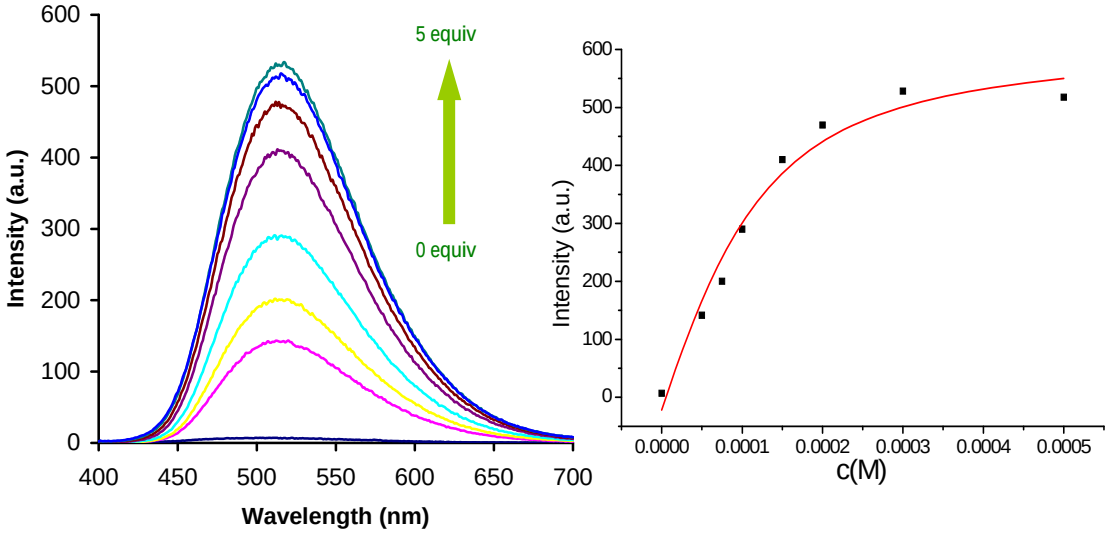
Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with tyramine in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 517 nm) for the titration of **3** with tyramine.



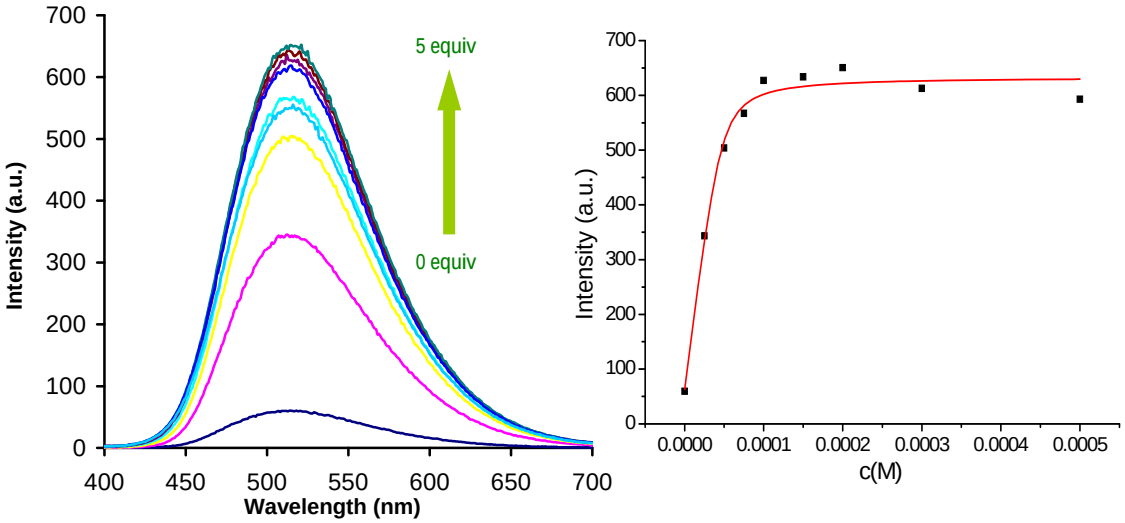
Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with putrescine in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 517 nm) for the titration of **3** with putrescine.



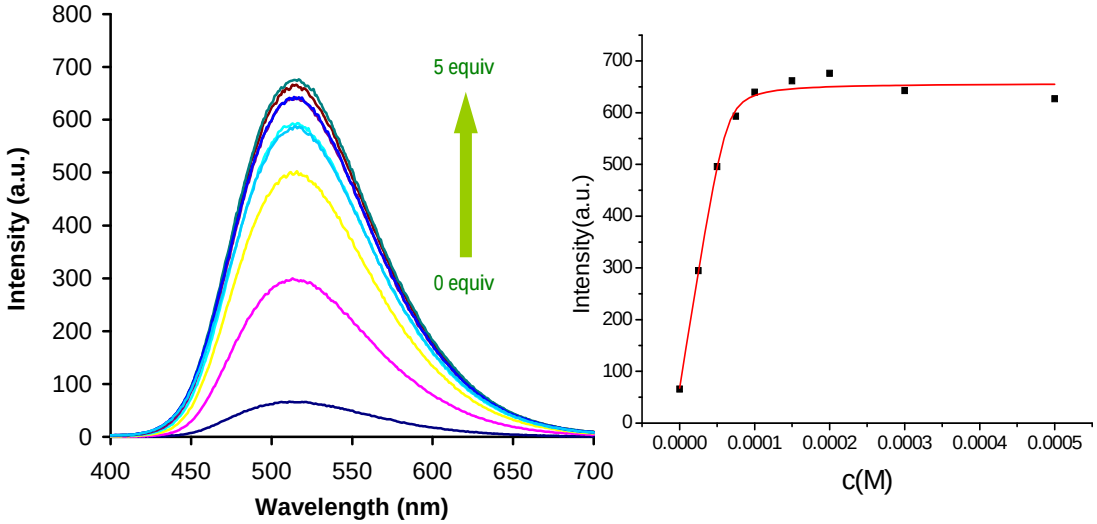
Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with cadaverine in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 517 nm) for the titration of **3** with cadaverine.



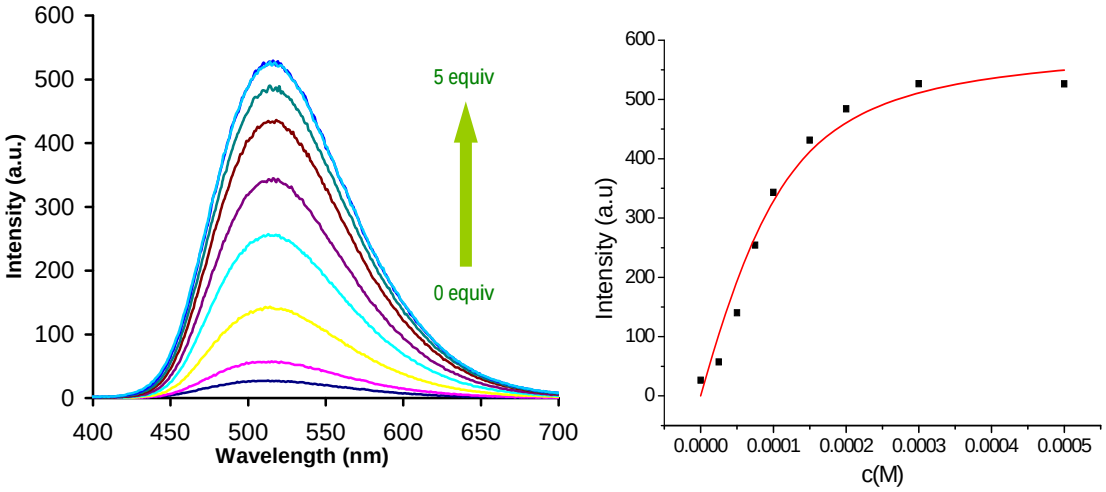
Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with cadaverine HEPES, pH 8.2 in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 517 nm) for the titration of **3** with cadaverine HEPES, pH 8.2.



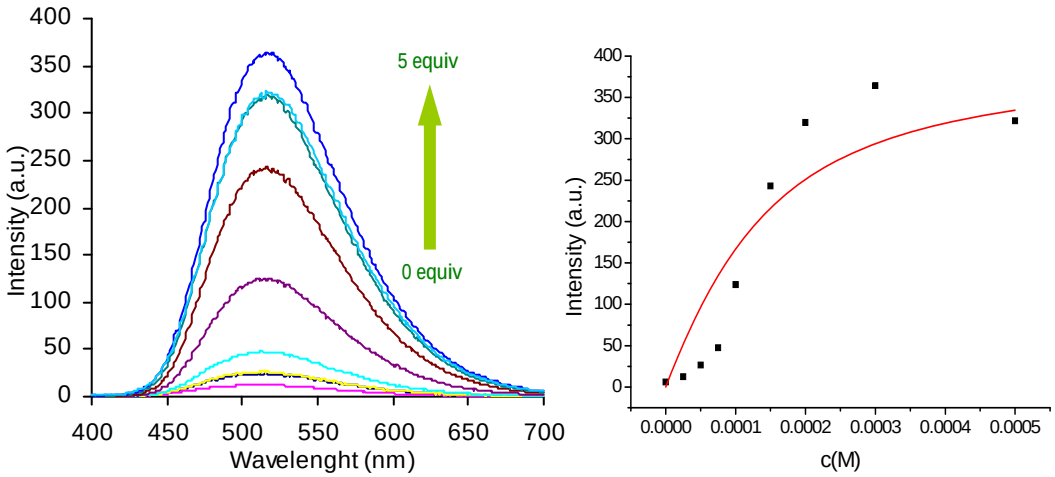
Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with spermidine in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 517 nm) for the titration of **3** with spermidine.



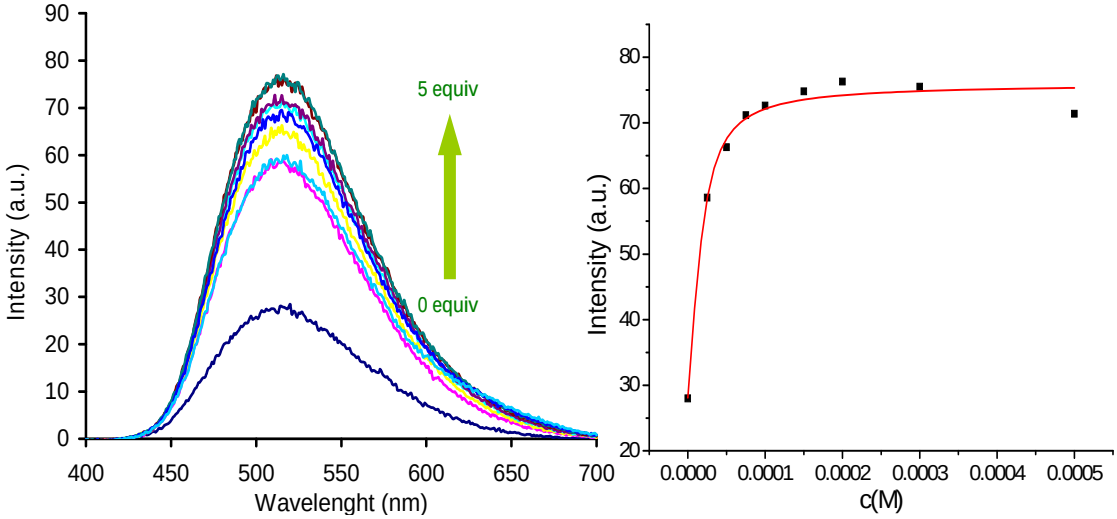
Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with spermine in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 517 nm) for the titration of **3** with spermine.



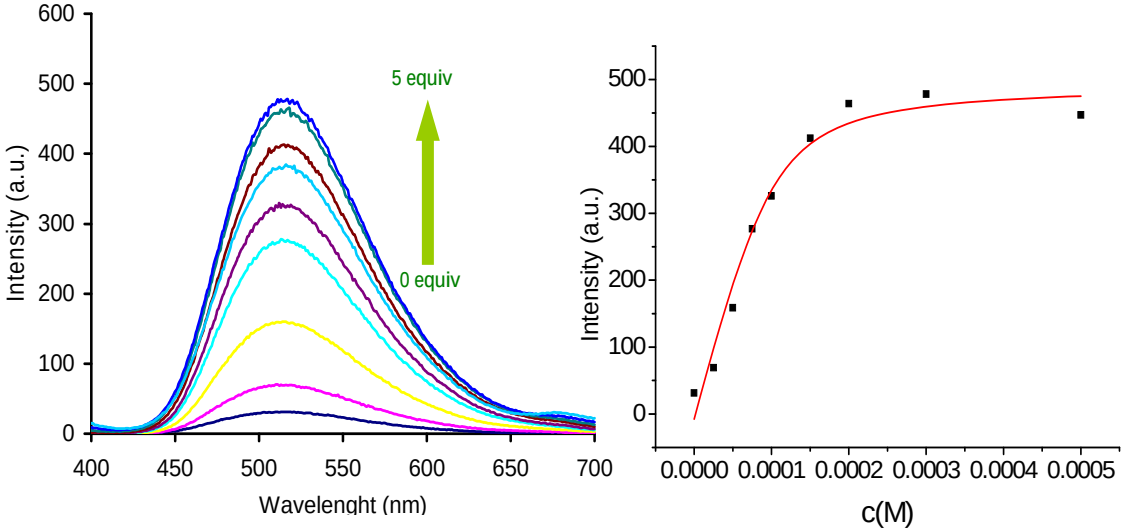
Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with hystamine in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 517 nm) for the titration of **3** with hystamine.



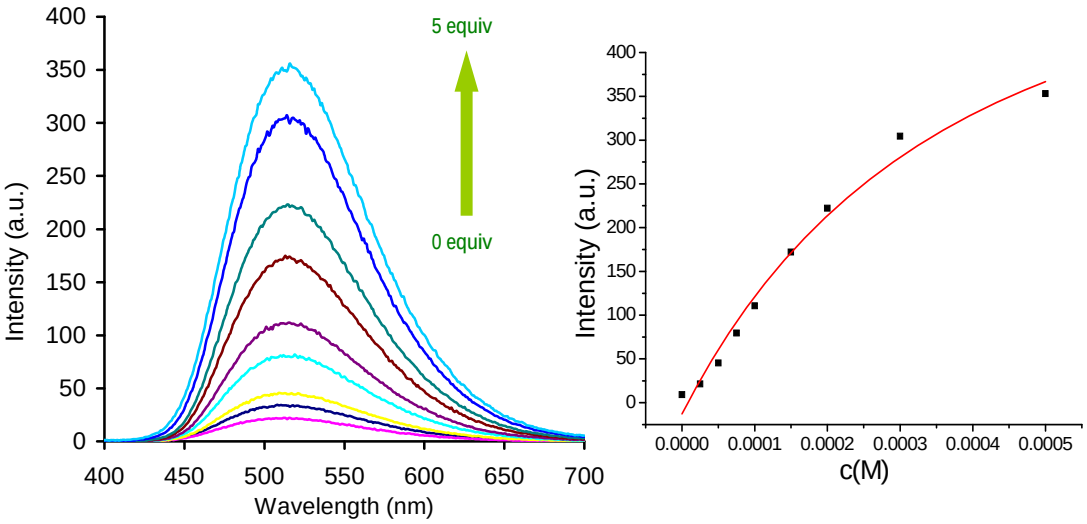
Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with dopamine in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 517 nm) for the titration of **3** with dopamine.



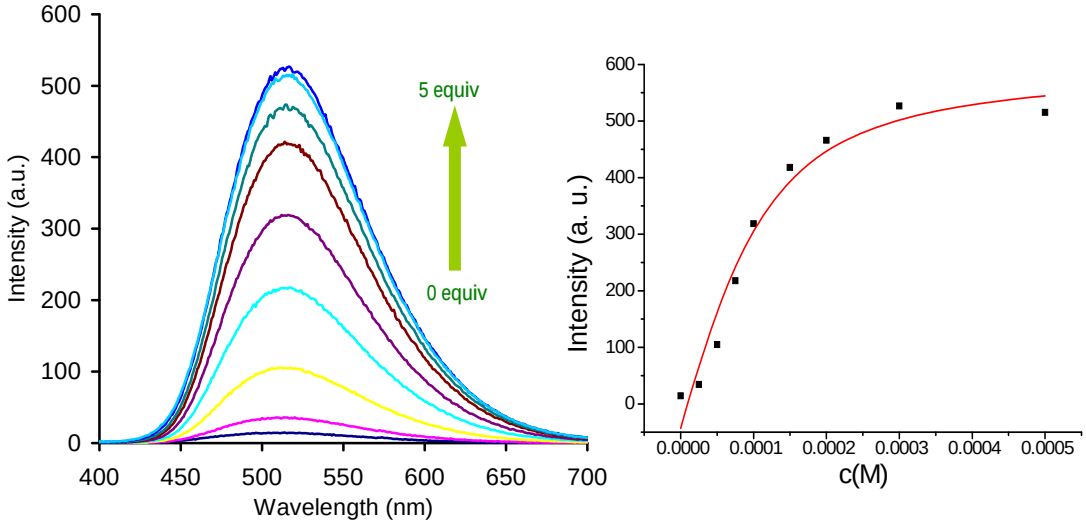
Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with dopamine HEPES, pH 8.2 in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 517 nm) for the titration of **3** with dopamine HEPES, pH 8.2.



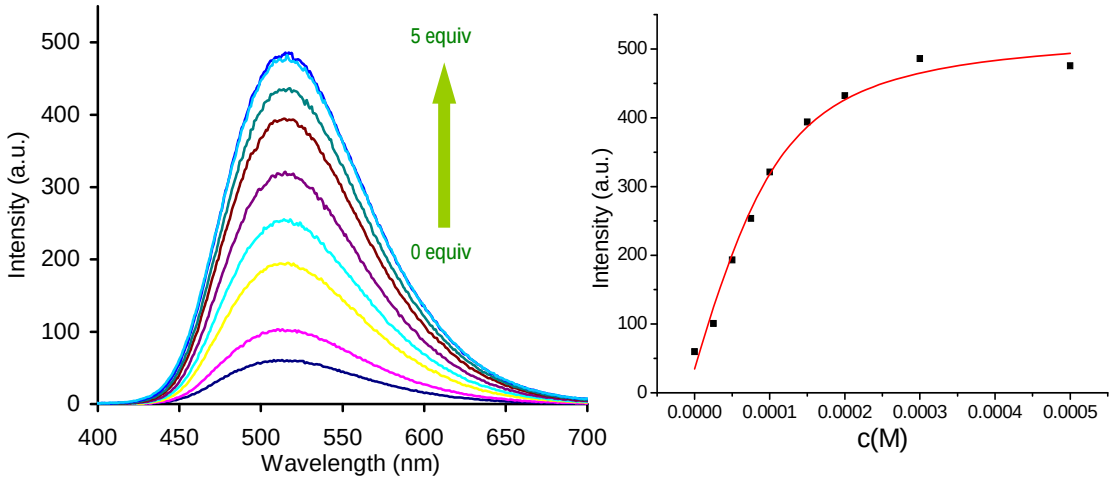
Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with serotonin in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 517 nm) for the titration of **3** with serotonin.



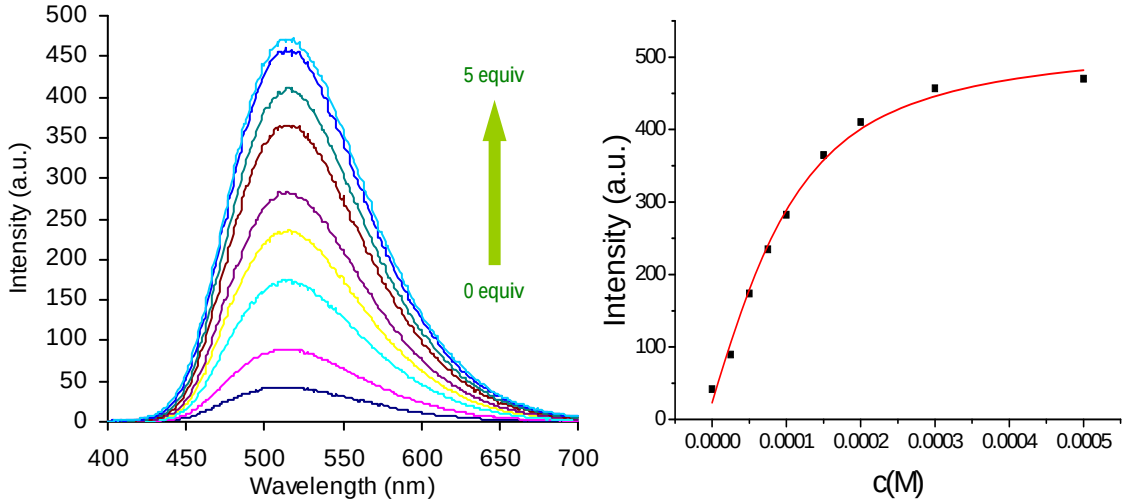
Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with cysteamine in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 517 nm) for the titration of **3** with cysteamine.



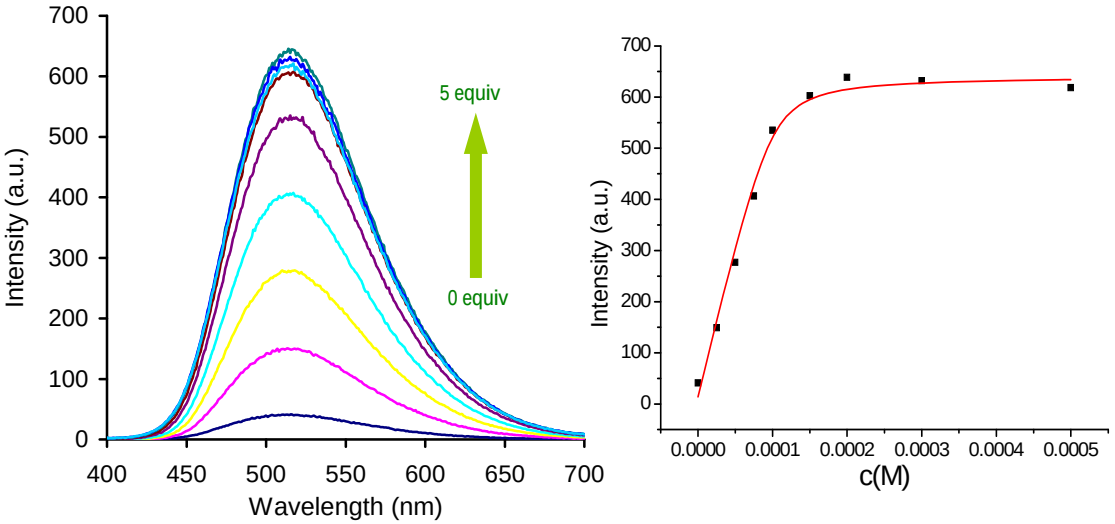
Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with ethanolamine in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 517 nm) for the titration of **3** with ethanolamine.



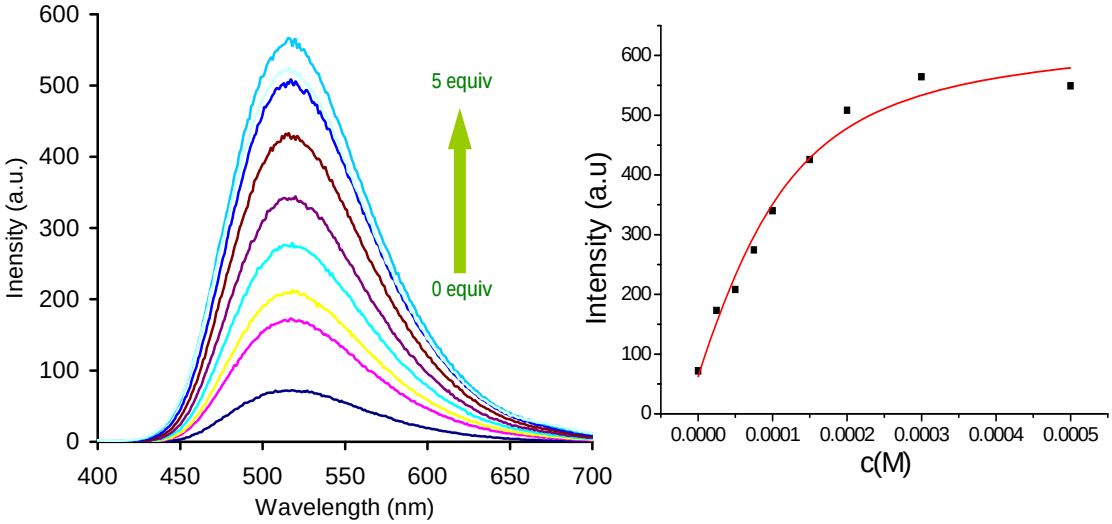
Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with ephedrine in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 515 nm) for the titration of **3** with ephedrine.



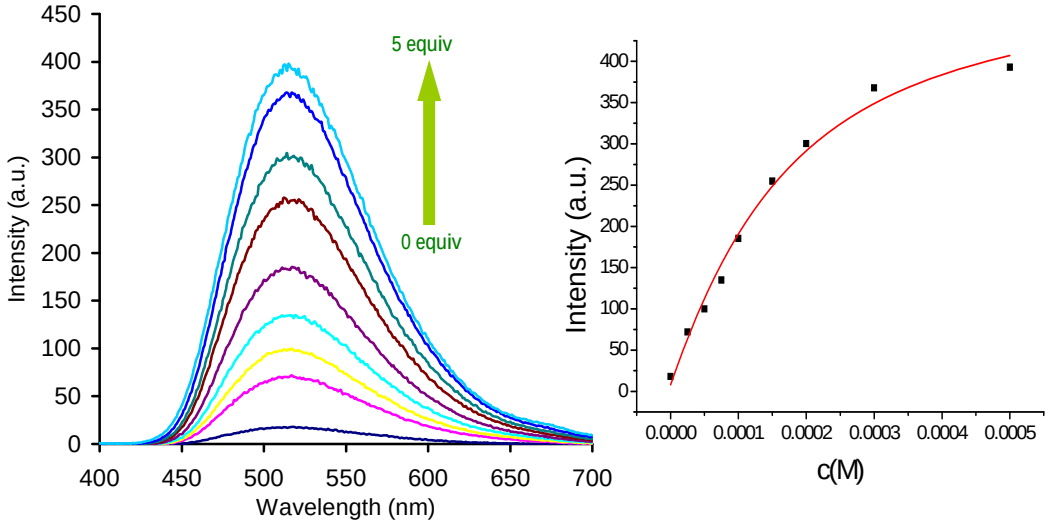
Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with pseudoephedrine in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 515 nm) for the titration of **3** with pseudoephedrine.



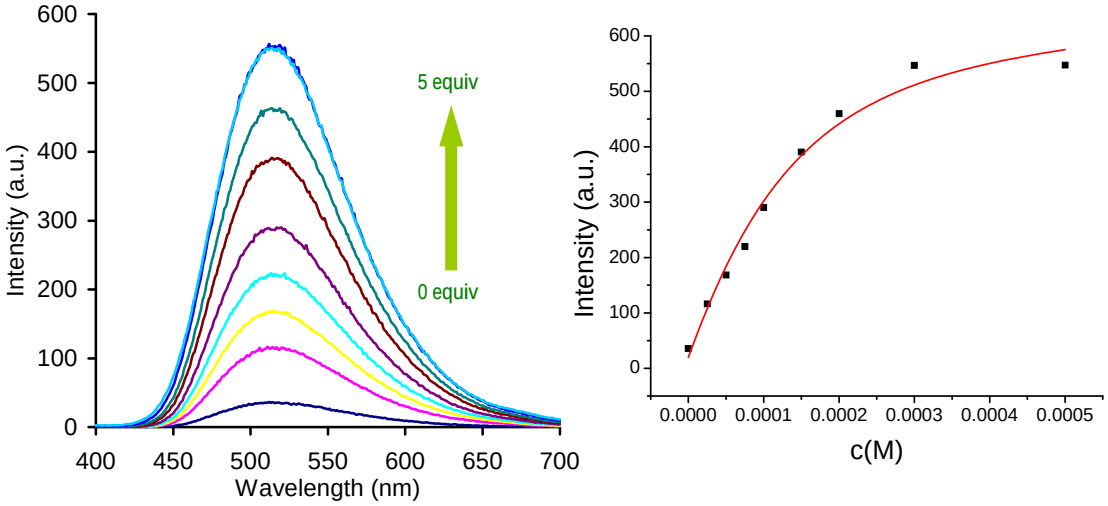
Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with amphetamine in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 515 nm) for the titration of **3** with amphetamine.



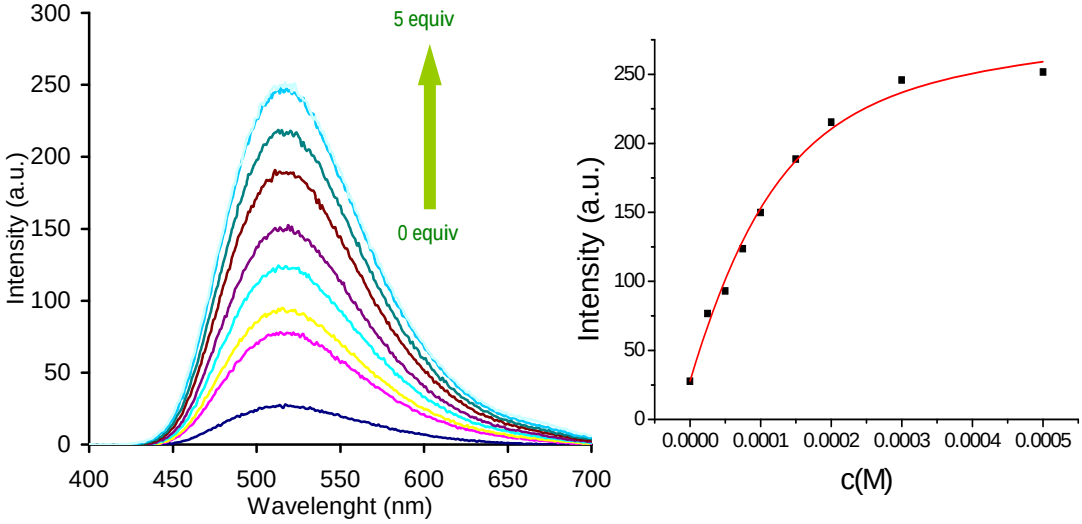
Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with MDA in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 517 nm) for the titration of **3** with MDA.



Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with MDA HEPES, pH 8.2 in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 517 nm) for the titration of **3** with MDA HEPES, pH 8.2.



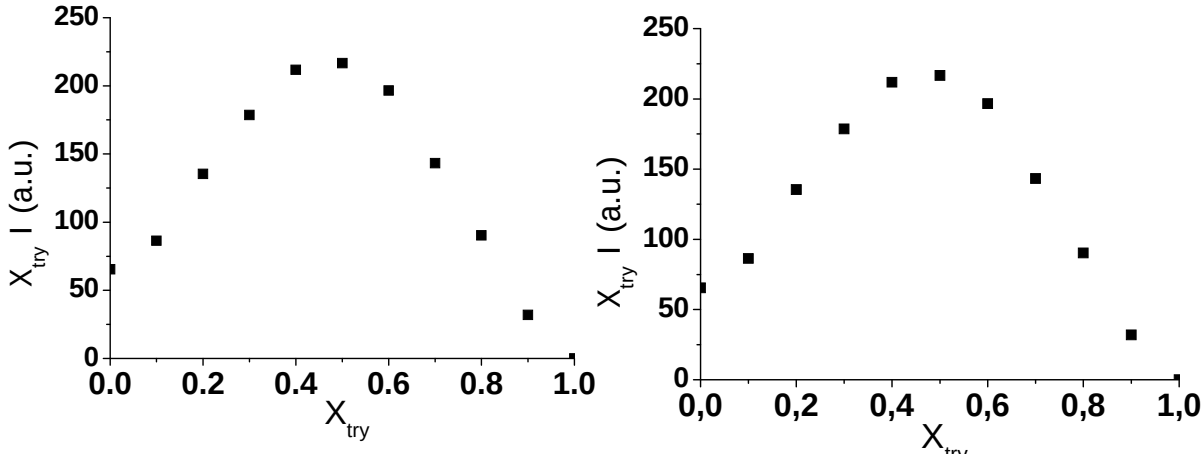
Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with MDMA in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 517 nm) for the titration of **3** with MDMA.



Left: Emission spectra for the fluorimetric titration of **3** (10^{-4} M in DMSO) with MDMA HEPES, pH 8.2 in water (λ_{exc} = 288 nm). Right: Titration profile fitted to a 1:1 model (λ_{max} = 517 nm) for the titration of **3** with MDMA HEPES, pH 8.2.

10.-Job's plot analysis of fluorescence titrations of 3 with triptamine or spermine

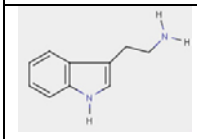
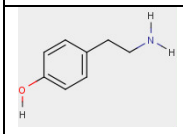
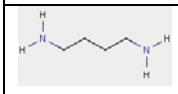
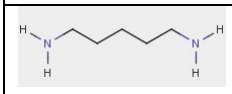
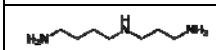
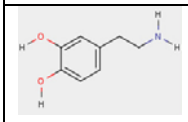
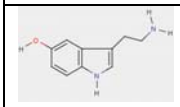
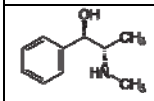
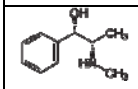
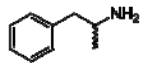
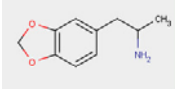
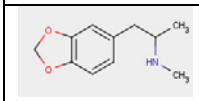
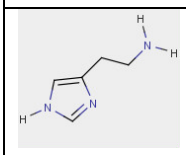
Job's plot analysis of fluorescence titrations of **3** with triptamine or spermine revealed maximum peaks at 50% mole fraction, in accord with the proposed 1:1 binding stoichiometry



Left: Job's plot analysis of fluorescence titrations of **3** (10^{-4} M in DMSO) with triptamine in water (λ_{exc} = 288 nm) (λ_{em} = 517 nm), T = 25 ºC. Right: Job's plot analysis of fluorescence titrations of **3** (10^{-4} M in DMSO) with spermine in water (λ_{exc} = 288 nm) (λ_{em} = 517 nm) , T = 25 ºC.

11.- CALCULATION OF THE ACIDIC DISSOCIATION CONSTANTS OF BIOGENIC AMINES

Table 10-1.- Acidic Dissociation Constants, pK_i (i = 1,2), of different amines in water (refs. *a* to *g*), and in 80/20 DMSO/H_2O from measures in 40/60 DMSO/H_2O (ref. *h*) and in 80/20 DMSO/H_2O (i, calculated from pK_i in water). T= 25°C.

Structure	Name	pK_1	pK_2	$pK_{DMSO/water}$ [h]
	Triptamine		10.2[a]	11.0
	Tyramine	9.74[a] 10.02±0.05[h]	10.52[a] 10.79±0.06[h]	11.3
	Putrescine	9.63 [a]	10.8[a]	10.4
	Cadaverine	10.05 [a]	10.93 [a]	10.8
	Spermidine	10.80[b]	11.56[b]	11.6
	Spermine	10.95[b]	11.50[b]	11.7
	Dopamine	8.9 [a] 9.27±0.02[h]	10.6 [a] 10.9±0.6[h]	11.4
	Serotonine	9.8 [a]	11.1[a]	11.9
	Ephedrine		9.6[c]	10.4
	Pseudoephedrine		9.8[d]	10.6
	Amphetamine		9.8[e] 10.15±0.07[h]	10.6
	MDA		10.04[f]	10.8
	MDMA		10.38[g]	11.2
	Histamine	6.04 [a] 6.38±0.04[h]	9.75 [a] 10.05±0.07[h]	10.5

a) A) Handbook of Chemistry and Physics: http://www.hbcpnetbase.com/ B) Handbook of Chemistry and Physics, David R. Lide, Ed., 90ND Edition 2009-2010.

[b] Y. Takeda; K. Samejima; K. Nagano; M. Watanabe; H. Sugeta; Y. Kyogoku, *Eur. J. Biochem.,* **1983**, *130*, 383-389, (pK of NH_2 groups).

[c] P. Jankovics, T. Nemeth, J. Nemeth-Palotas, H. Koszegi-Szalai, *Acta Chromatogr.* **2010**, *224*, 527-538.

[d] A. C. Moffat, in *Clarcke's isolation and identification of drugs*, *The Pharmaceutical Press*, London, **1986**, p. 520.

[e] F. Tagliaro; F. P. Smith; S. Turrina; V. Equisetto; M. Marigo, *J. Chromatogr. A* **1996**, *735*, 227-235.

[f] R. C. Clarka, A. K. Valaera, W. R. Ravisa, *J. Liq. Chromatogr. R. T.* **1990**, *13*, 1375-1385.

[g] E. A. De Letter; K. M. Clauwaert; F. M. Belpaire; W. E. Lambert; J. F. Van Bocxlaer; M. H. A. Piette, *Int. J. Legal Med.* **2002**, *116*, 216-224.

[h] This work, from measures in 40/60 and 80/20 w/w DMSO/H_2O

Materials and methods

We used commercial or synthesized amines, commercial DMSO (Panreac PA) and bidistilled water. The mixture of solvents was prepared by weighting in a Sartorius CP225D precision balance. NaOH solution in DMSO/H_2O was titrated with potassium phthalate. Potentiometric titration was performed with a Metrohm 716 DMS Titrino titrator. pH-meter was calibrated with 6 buffer solutions, pH between 7.00 and 12.45. All experiments were performed at 25º C by means a Julabo F34 circulator bath.

Acidity dissociation constants, pK_i, in 40% and 80% w/w DMSO/H_2O. T = 25ºC

The calculation of acidity dissociation constants of amines in 80/20 w/w DMSO/H_2O was performed by two different methods, spectrophotometric and potentiometric. The first was not suitable in most cases because the absorption bands of amine and DMSO overlapped. In addition, potentiometric methods are not suitable for 80% DMSO solutions because addition of base (NaOH 0.1 M) gives rise to a biphasic system before the titration is concluded. Therefore we used potentiometric methods in 40/60 w/w DMSO/H_2O, which is the maximum concentration of DMSO to have a monophasic system. Because the pH-meter was calibrated with buffer solutions in water, the pH value was corrected by applying:

$$pH = pH_R - \delta \qquad [1]$$

Where pH_R is the average value, pH the corrected value and δ the correction factor, with values of 0.3 and 0.8 for 40/60 w/w DMSO/H_2O and 80/20 w/w DMSO/H_2O, respectively. (D. D. Perrin, B. Dempsey "Buffers for pH and Metal Ion Control" Chapman and Hall, 1974, p. 79).

Overlapping equilibria: Dopamine (40% w/w DMSO/H_2O):

As can be seen in table 10-1, several amines show two close constants of acidic dissociation K_{a1} and K_2 ($\Delta pK < 3$). For example, figure 10-1 shows potentiometric titration of dopamine, in which two partially overlapped equilibria are observed, and the incomplete resolution of the first one, therefore it was necessary to apply specific methods for the calculation of constants for partially overlapped equilibria. In this case we used the method of Speakman (A. Albert and E.P. Serjeant. "The determination of ionization constants. A laboratory Manual" 3 Ed. Chapman and Hall 1984, p. 56).

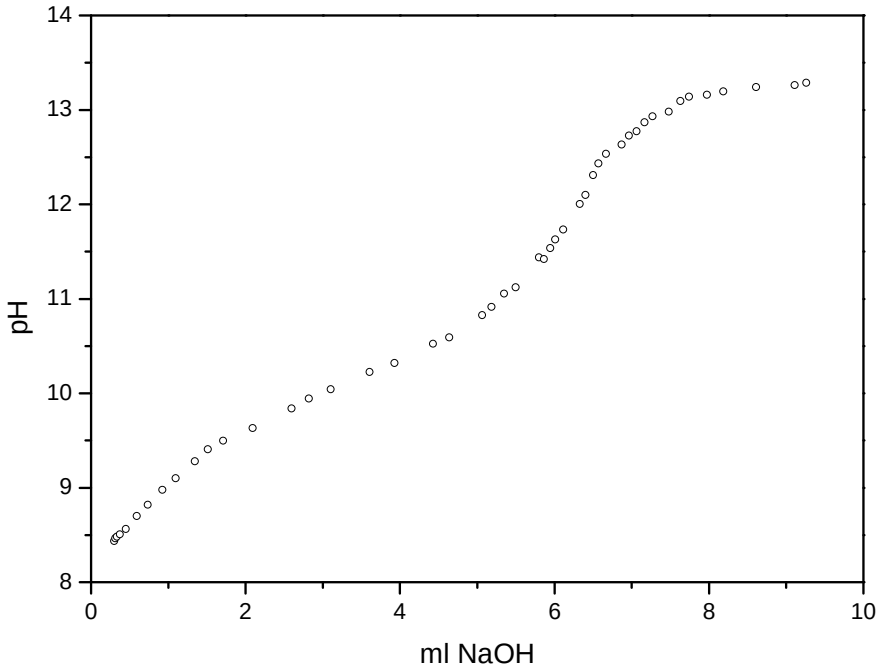


Figure 10-1: Potentiometric titration of Dopamine. [Dopamine] = 0,05M, [NaOH] = 0.17 M. 40/60 w/w DMSO/ H_2O. T=25°C.

In the case of dopamine, with two acidic hydrogens, the dissociation equilibria can be represented by:

$$(2)\quad H_2A \overset{K_1}{\rightleftharpoons} H^+ + HA^-$$

$$(3)\quad HA^- \overset{K_2}{\rightleftharpoons} H^+ + A^{2-}$$

Where H_2A, HA^- and A^{2-} represent dopamine as the neutral, mono and dianionic species, respectively, and K_1 and K_2 the acidic dissociation constants for H_2A and HA^- respectively. With the mass and charge balances and the definitions of K_1 and K_2, we obtain equation [4]

$$\frac{[H^+]^2 F}{K_1(2-F)} - K_2 = [H^+]\frac{1-F}{2-F} \qquad (4)$$

Where

$$F = \frac{C_A + [H^+] + [OH^-]}{C} \qquad (5)$$

In Eq 5, $C = [H_2A]_o V/(V_{NaOH}+V)$ and $C_A = [NaOH]V_{NaOH}/(V_{NaOH}+V)$ are the concentrations of H_2A and NaOH, respectively. The procedure for the calculation of K_1 and K_2 is iterative. From the corrected pH the concentrations of $[H^+]$ and $[OH^-]$ are obtained; with them, F was determined as a function of $[H^+]$ (Eq 5). With Eq 4, by means of least square fitting, the initial values of K_1 and K_2 are calculated and, from them, the concentrations of all species from equilibria (2) and (3), the ionic strength of the solution, the activity coefficients of H^+ and OH^-, and their activities are estimated. With the activities of H^+ and OH^-, F was calculated by using Eq. 4, and used in Eq. 5. The process is repeated until convergence of K_1 and K_2. In general, less than

five iterations are needed to get convergence. In this way, the values obtained for dopamine are: $pK_1 = 9.27 \pm 0.02$ and $pK_2 = 10.9 \pm 0.6$.

Following the same procedure we calculated the pK values of tyramine and hystamine. The amphetamine constant was calculated by a simpler procedure because there is only one ionizable group (table 1). In all cases, even considering the error limits, the pK value in the mixture of 40% w/w DMSO/water is different of the pK in water by approximately 0.3 units, which is the value of δ for this mixture in eq (1). By extrapolation of these results the estimation of pK_{DMSO} in 80% w/w DMSO/H_2O should be possible, by addition of the correction value $\delta = 0.8$. (see previous paragraph) to the pK_i values in water (table 1). In summary, the presence of DMSO increases the basicity of functional groups. In cases where there are two or more functional groups, such as putrescine, cadaverine, spermidine and spermine, we have taken $pK_{DMSO} = pK_1 + 0.8$ for calculation of pK in DMSO/H_2O 80% w/w.

In cases where there are two different functional groups, OH and $–NH_2$, such as dopamine and serotonin, pK_1 corresponds to dissociation of the -OH group and pK_2 to dissociation of the $–NH_2$ group. Assignation was performed by taking in account that the phenol acidic constant pK = 9.99 (ref. Handbook) was lower than the tryptamine acidic constant $pK_2 = 10.2$. In the case of histamine there is a large difference between the values of pK, indicating that pK_2 corresponds to the dissociation of the primary amine. For the rest of amines, pK_2, corresponds to dissociation of amine group. In all cases the equilibrium constant in DMSO/H_2O w/w 80% was $pK_{DMSO} = pK_2 + 0.8$. In table 1 the approximate values of pK_{DMSO} in the experimental conditions are shown.

12.- PRINCIPAL COMPONENTS ANALYSIS

Analysis Summary:

Data variables: pK_{DMSO}, $logK_{eq}$, Kv_{form}, $Kv_{quenching}$,

	Col_1	Col_2	Col_3	Col_4
	AMINE	**pK_{DMSO}**	**logKeq**	**Kv formation**	**Kv quenching**
1	Tryptamine	11.0	5.40	0.04776	0.00444705
2	Tyramine	11.3	5.17	0.02652	0.00216798
3	Putrescine	10.4	5.49	0.09082	0.00813422
4	Cadaverine	10.8	6.23	0.06433	0.00787294
5	Spermidine	11.6	5.48	0.092402	0.0071713
6	Spermine	11.7	5.78	0.08718	0.00752218
7	Dopamine	11.4	4.09	0.04776	0.00444705
8	Serotonin	11.9	4.83	0.02652	0.00216798
9	Ephedrine	10.4	4.50	0.06545	0.0054153
10	Pseudoephedrine	10.6	4.36	0.07158	0.00573527
11	Amphetamine	10.6	5.35	0.02443943	0.0072249
12	MDA	10.8	3.95	0.03658	5.32E-04
13	MDMA	11.2	4.23	0.135	4.42E-05

Data input: observations. Number of complete cases: 13. Missing value treatment: listwise. Standardized: yes

Number of components extracted: 2

Principal Components Analysis:

COMPONENT NUMBER	EIGENVALUE	PERCENT OF VARIANCE	CUMULATIVE PERCENTAGE
1	1.71557	42.889	42.889
2	1.16789	29.197	72.086
3	0.847607	21.190	93.277
4	0.268935	6.723	100.000

This procedure performs a principal components analysis. The purpose of the analysis is to obtain a small number of linear combinations of the 4 variables which account for most of the variability in the data. In this case, 2 components have been extracted, since 1888 components had eigenvalues greater than or equal to 1.0. Together they account for 72.1% of the variability in the original data.

Table of Component Weights:

	COMPONENT 1	COMPONENT 2
Col_1	0.0433929	0.731311
Col_2	0.697214	-0.0525439
Col_3	0.146357	0.667008
Col_4	0.700421	-0.132379

This table shows the equations of the principal components.

First principal component has the equation:

$0.0433929 * pK_{DMSO} + 0.697214 * logK_{eq} + 0.146357 * Kv_{formation}\ 3 + 0.700421 * Kv_{quenching}$

The second principal component:

$0.731311 * pK_{DMSO} - 0.0525439 * logK_{eq} + 0.667008 * Kv_{formation}\ 3 - 0.132379 * Kv_{quenching}$

Where the values of the variables in the equation are standardized by subtracting their means and dividing by their standard deviations.

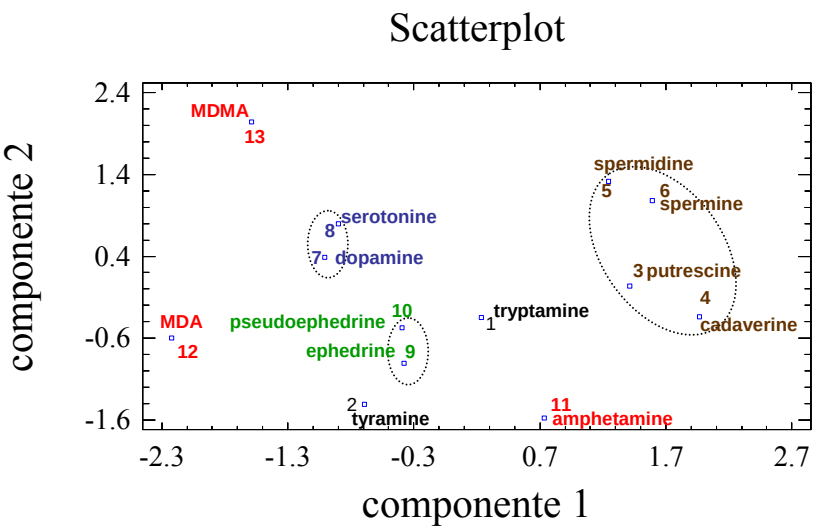
With these new equations we calculated a system of two coordinates formed by the components 1 and 2.

Table of Principal Components:

	Component 1	Componente 2
1	0.235572	-0.344579
2	-0.694747	-1.41377
3	1.41245	0.0390241
4	1.96394	-0.33861
5	1.2422	1.31926
6	1.59326	1.07918
7	-1.00649	0.384509
8	-0.897348	0.793917
9	-0.380362	-0.911412
10	-0.39149	-0.474585
11	0.733849	-1.5801
12	-2.22096	-0.598927
13	-1.58987	2.04609

This table shows the values of the principal components for each row of the data file.

Graphical plot of the table of principal components:



Analysis of results:

The distribution of the points in the representation of the two components show the next features:

a) Four points (3, 4, 5, 6) are separated from the rest of points and are characterized by high values of the component 1, in this component the variable imputs have an important correlation pK_{DMSO}, Kv formation and Kv quenching, and these amines have the most elevated value of these variable inputs, at the same time they have low values of the component 2, in which the most important negative correlation is given for the variable inputs logKeq y Kv quenching.

b) Another group is also separated from the rest, points 1, 2, 9, 10, which are in the central part of the graphic plot. These points show negative values of the component 1, pKa, Kv formation and Kv quenching, lower than the values of other amines. The results are similar for 10 and 9.

c) Points 8 and 7 show similar values, negative with respect to the component 1 and positive with respect to the component 2, therefore they are characteristically separated from the rest.

d) Points 13, 12 and 11, which are derivatives of amphetamine, are separated from the rest because of the high value of the component 2. This component has a strong positive correlation with the variable input Kv formation, therefore the amine 13, for example, which shows a high value in the formation speed constant, is placed in a separate place in the representation.

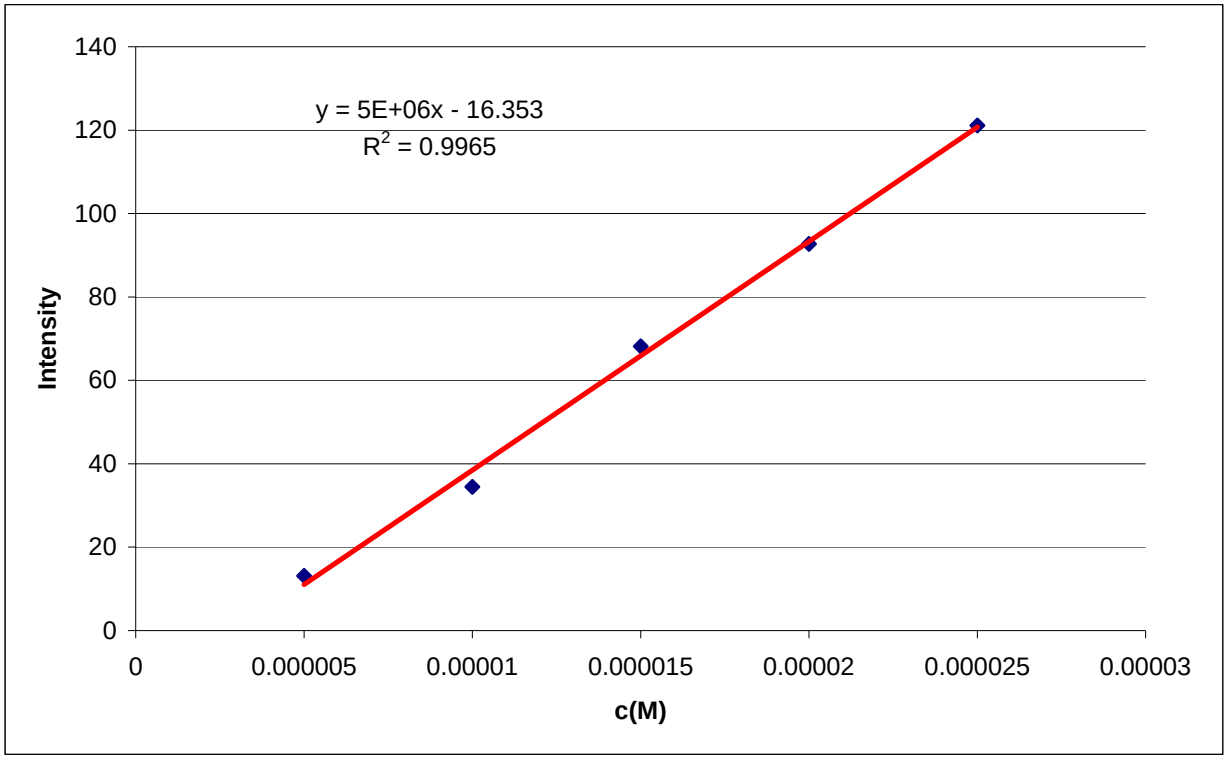
13.-CALCULATION OF DETECTION LIMITS

Calculation of the Detection Limit of 6 in DMSO + cadaverine in water

In a typical example, the detection limit was performed by using the two solvents previously used for the rest of essays, DMSO for the solution of **3** and water for the solution of cadaverine. The method used for the calculations is the blank variability as described in the *Handbook of Chemometrics and Qualimetrics*[1]. For this purpose, first a calibration plot is performed, then it is validated and then, in the same conditions, several blank replicates are measured. In this case, the solution of **3** gives a signal without the presence of the analyte, therefore this signal is taken as the blank; 10 replicates of the blank were measured. A calibration with 5 measures and 10 replicates for the blank were taken from a 10^{-4} M solution of **6**:

c(M) cadaverine	Intensity
0.000005	13.12966537
0.00001	34.44453812
0.000015	68.186203
0.00002	92.74793243

With these values the calibration curve was drawn:



The adjusted equation by least squares is obtained:

[1] D. L. Massart, B. G. M. Vandeginste, L. M. C. Buydens, S. De Jong, P. J. Lewi, J. Smeyers-Verbeke, *Handbook of Chemometrics and Qualimetrics: Part A*, Eselvier, Amsterdan, The Netherlands, **1997**, Chapt. 13, 379.

$$y = 5E + 06x - 16.353 \quad [1]$$

Then, 10 replicates for the blank were taken. The measured blank emission values, from a solution of **3**, and the values of the concentration calculated from equation [1] are detailed.

replicates	Intensity
1	3.709601879
2	3.645466328
3	3.773766994
4	3.78967905
5	3.679283142
6	3.70250082
7	3.766991138
8	3.859928131
9	3.689877987
10	3.888637543

The mean ($\bar{x}$) and the standard deviation (s) from the fluorescence data are:

$$\bar{x} \pm s = (3.75 \pm 0.08) \quad [2]$$

For a probability level of 5% (α=β=0.05), the value of t_C=t_D from bibliography,[1] for 9 freedom degrees ($GL = N - 1 = 10 - 1 = 9$) is 1.833.

Therefore, the decision limit is given by the equation:

$$L_C = t_C \times s \times \sqrt{1 + \frac{1}{N}} \quad [3]$$

N is the number of blank replicates (10). Therefore:

$$L_C = t_C \times s \times \sqrt{1 + \frac{1}{N}} = 1.833 \times 0.08 \times \sqrt{1 + \frac{1}{10}} = 0.153$$

The detection limit corresponds to the double of the decision limit, consequently:

$$L_D = 2L_C = 0.306$$

The detection limit is given in terms of concentration by:

$$x_D = 2x_C = \frac{2L_C}{b} \quad [4]$$

Where b is the rate of the calibration plot [1]. Therefore:

$$x_D = \frac{2L_C}{b} = \frac{2 \times 0.153}{5E-06} = 6.128 \cdot 10^{-8}$$

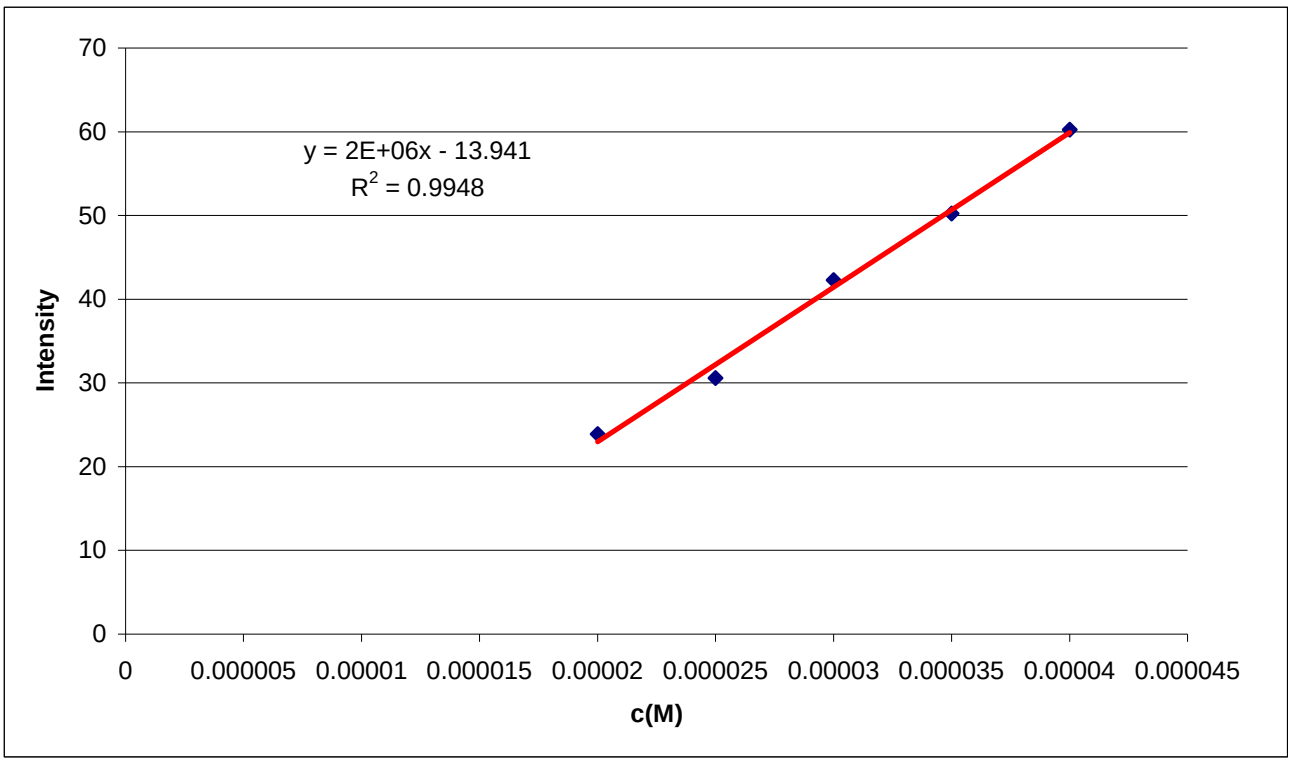
Consequently, the detection limit of **3** at a concentration of $C_6 = 10^{-4}$ M and cadaverine in water, by fluorescence measures, corresponds to $6.128 \cdot 10^{-8}$ **M.**

Calculation of the Detection Limit of 3 in DMSO + cadaverine in HEPES 8.2

The detection limit was performed by using two solvents, DMSO for the solution of **3** and HEPES pH 8.2 for the solution of cadaverine. The method used for the calculations is the blank variability as described in the *Handbook of Chemometrics and Qualimetrics*[2]. For this purpose, first a calibration plot is performed, then it is validated and then, in the same conditions, several blank replicates are measured. In this case, the solution of **3** gives a signal without the presence of the analyte, therefore this signal is taken as the blank; 10 replicates of the blank were measured. A calibration with 5 measures and 10 replicates for the blank were taken from a 10^{-4} M solution of **3**:

c(M) cadaverine	Intensity
0.00002	23.90303612
0.000025	30.59812737
0.00003	42.27688599
0.000035	50.23363876

With these values the calibration curve was drawn:



[2] D. L. Massart, B. G. M. Vandeginste, L. M. C. Buydens, S. De Jong, P. J. Lewi, J. Smeyers-Verbeke, *Handbook of Chemometrics and Qualimetrics: Part A*, Eselvier, Amsterdan, The Netherlands, **1997**, Chapt. 13, 379.

The adjusted equation by least squares is obtained:

$$y = 2E + 06x - 13.941 \quad [1]$$

Then, 10 replicates for the blank were taken. The measured blank emission values, from a solution of **3**, and the values of the concentration calculated from equation [1] are detailed.

replicates	Intensity
1	8.27958489
2	7.77818108
3	7.25698233
4	7.53585625
5	7.05536032
6	7.06559038
7	6.77952576
8	7.03823662
9	6.84187269
10	6.72350645

The mean ($\bar{x}$) and the standard deviation (s) from the fluorescence data are:

$$\bar{x} \pm s = (7.235 \pm 0.494) \quad [2]$$

For a probability level of 5% (α=β=0.05), the value of t_C=t_D from bibliography,[1] for 9 freedom degrees ($GL = N - 1 = 10 - 1 = 9$) is 1.833.

Therefore, the decision limit is given by the equation:

$$L_C = t_C \times s \times \sqrt{1 + \frac{1}{N}} \quad [3]$$

N is the number of blank replicates (10). Therefore:

$$L_C = t_C \times s \times \sqrt{1 + \frac{1}{N}} = 1.833 \times 0.494 \times \sqrt{1 + \frac{1}{10}} = 0.950$$

The detection limit corresponds to the double of the decision limit, consequently:

$$L_D = 2L_C = 1.901$$

The detection limit is given in terms of concentration by:

$$x_D = 2x_C = \frac{2L_C}{b} \quad [4]$$

Where b is the rate of the calibration plot [1]. Therefore:

$$x_D = \frac{2L_C}{b} = \frac{2 \times 0.950}{2\text{E-}06} = 9.506 \cdot 10^{-7}$$

Consequently, the detection limit of **3** at a concentration of C_6 = 10^{-4} M and cadaverine in HEPES 8.2, by fluorescence measures, corresponds to $9.506 \cdot 10^{-7}$ **M.**

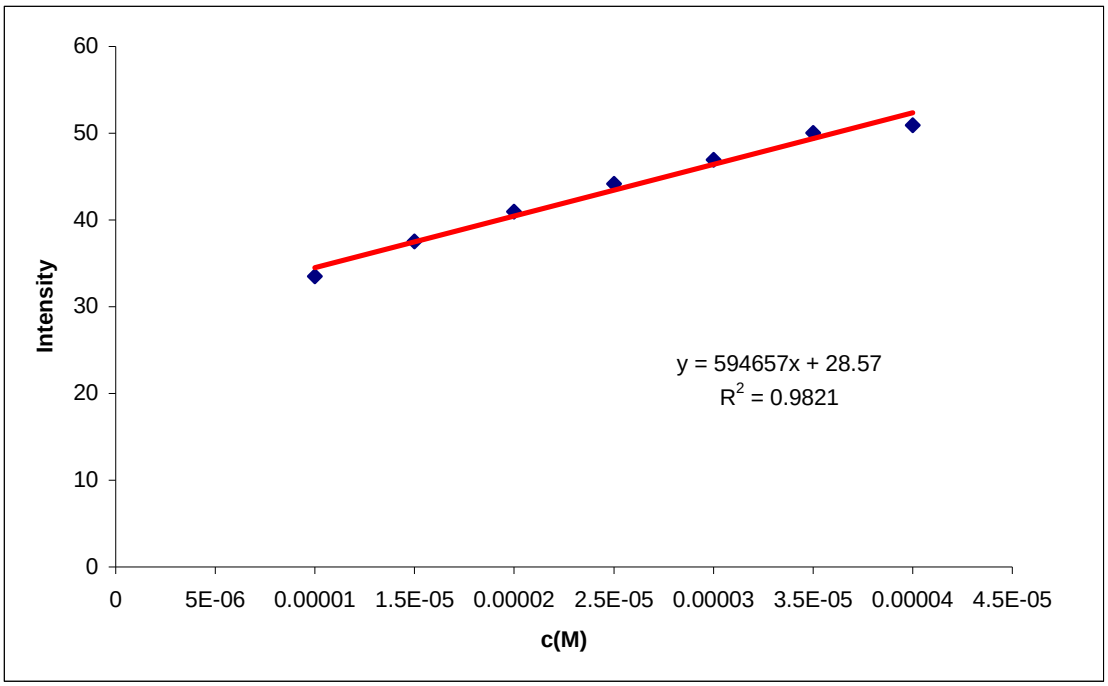
Calculation of the Detection Limit of 3 in DMSO + dopamine in HEPES 8.2

The detection limit was performed by using two solvents, DMSO for the solution of **3** and HEPES pH 8.2 for the solution of dopamine. The method used for the calculations is the blank variability as described in the *Handbook of Chemometrics and Qualimetrics*[3]. For this purpose, first a calibration plot is performed, then it is validated and then, in the same conditions, several blank replicates are measured. In this case, the solution of **3** gives a signal without the presence of the analyte, therefore this signal is taken as the blank; 10 replicates of the blank were measured. A calibration with 5 measures and 10 replicates for the blank were taken from a 10^{-4} M solution of **3**:

c(M) dopamine	Intensity
0.00001	33.51567459
0.000015	37.51980972
0.00002	40.95321274
0.000025	44.16679764
0.00003	46.94160461
0.000035	50.02903366
0.00004	50.93070602

With these values the calibration curve was drawn:

[3] D. L. Massart, B. G. M. Vandeginste, L. M. C. Buydens, S. De Jong, P. J. Lewi, J. Smeyers-Verbeke, *Handbook of Chemometrics and Qualimetrics: Part A*, Eselvier, Amsterdan, The Netherlands, **1997**, Chapt. 13, 379.



The adjusted equation by least squares is obtained:

$$y = 594657x + 28.57 \qquad [1]$$

Then, 10 replicates for the blank were taken. The measured blank emission values, from a solution of **3**, and the values of the concentration calculated from equation [1] are detailed.

replicates	Intensity
1	12.8386421
2	12.5114698
3	11.7736311
4	11.1278172
5	10.7912312
6	10.5880127
7	11.0851774
8	10.3444223
9	9.85433769
10	10.154727

The mean ($\bar{x}$) and the standard deviation (s) from the fluorescence data are:

$$\bar{x} \pm s = (11.106 \pm 0.991) \qquad [2]$$

For a probability level of 5% (α=β=0.05), the value of t_C=t_D from bibliography,[1] for 9 freedom degrees ($GL = N - 1 = 10 - 1 = 9$) is 1.833.

Therefore, the decision limit is given by the equation:

$$L_C = t_C \times s \times \sqrt{1+\frac{1}{N}} \qquad [3]$$

N is the number of blank replicates (10). Therefore:

$$L_C = t_C \times s \times \sqrt{1+\frac{1}{N}} = 1.833 \times 0.991 \times \sqrt{1+\frac{1}{10}} = 1.905$$

The detection limit corresponds to the double of the decision limit, consequently:

$$L_D = 2L_C = 3.811$$

The detection limit is given in terms of concentration by:

$$x_D = 2x_C = \frac{2L_C}{b} \qquad [4]$$

Where b is the rate of the calibration plot [1]. Therefore:

$$x_D = \frac{2L_C}{b} = \frac{2 \times 1.905}{594657} = 6.409 \cdot 10^{-6}$$

Consequently, the detection limit of **3** at a concentration of $C_6 = 10^{-4}$ M and dopamine in HEPES 8.2, by fluorescence measures, corresponds to $6.409 \cdot 10^{-6}$ **M.**

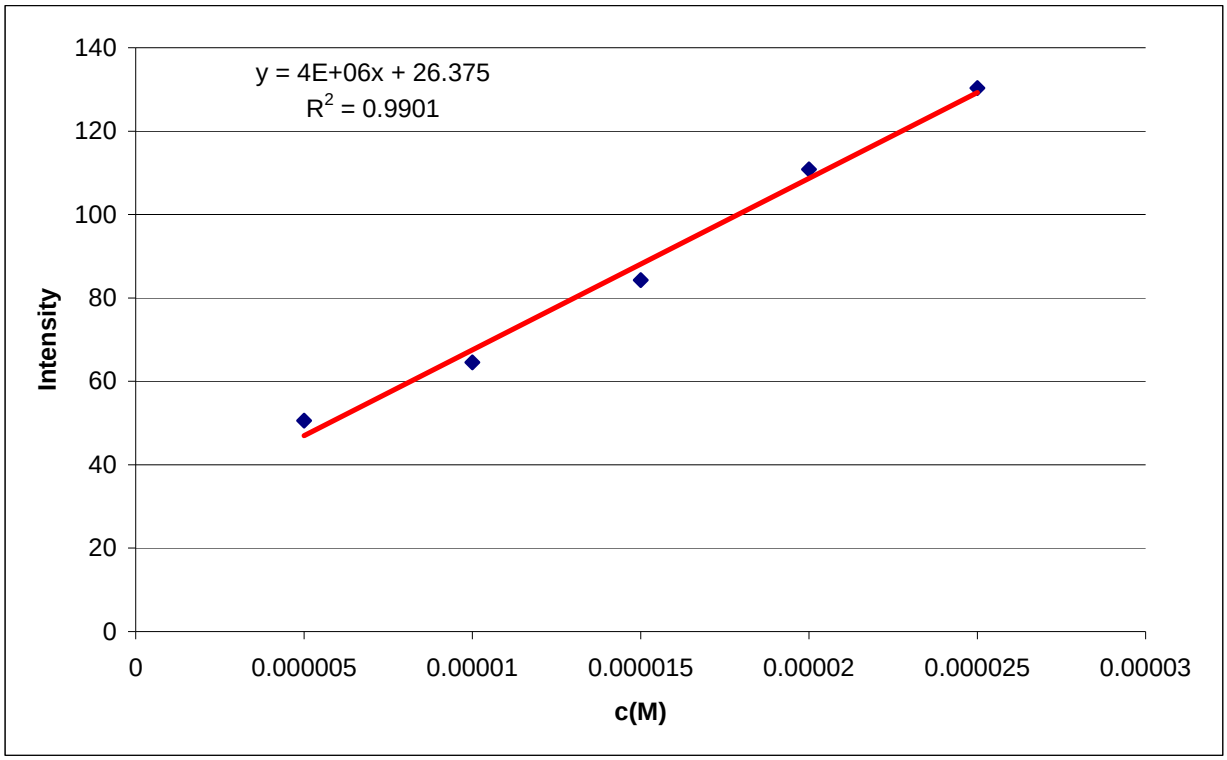
Calculation of the Detection Limit of 6 in DMSO + tryptamine in water

The detection limit was performed by using the two solvents previously used for the rest of essays, DMSO for the solution of **3** and water for the solution of tryptamine. The method used for the calculations is the blank variability as described in the *Handbook of Chemometrics and Qualimetrics*[4]. For this purpose, first a calibration plot is performed, then it is validated and then, in the same conditions, several blank replicates are measured. In this case, the solution of **3** gives a signal without the presence of the analyte, therefore this signal is taken as the blank; 10 replicates of the blank were measured. A calibration with 5 measures and 10 replicates for the blank were taken from a 10^{-4} M solution of **3**:

c(M) tryptamine	Intensity
0.000005	50.57216644
0.00001	64.58322144
0.000015	84.257164
0.00002	110.821022

[4] D. L. Massart, B. G. M. Vandeginste, L. M. C. Buydens, S. De Jong, P. J. Lewi, J. Smeyers-Verbeke, *Handbook of Chemometrics and Qualimetrics: Part A*, Eselvier, Amsterdan, The Netherlands, **1997**, Chapt. 13, 379.

With these values the calibration curve was drawn:



The adjusted equation by least squares is obtained:

$$y = 4E + 06x + 26.375 \qquad [1]$$

Then, 10 replicates for the blank were taken. The measured blank emission values, from a solution of **6**, and the values of the concentration calculated from equation [1] are detailed.

replicates	Intensity
1	33.4498177
2	33.1761856
3	33.8097458
4	32.5894585
5	33.0612259
6	32.1259499
7	31.4724674
8	31.8877544
9	31.3698807
10	31.7590008

The mean ($\bar{x}$) and the standard deviation (s) from the fluorescence data are:

$$\bar{x} \pm s = (32.478 \pm 0.868) \qquad [2]$$

For a probability level of 5% ($\alpha=\beta=0.05$), the value of $t_C=t_D$ from bibliography,[1] for 9 freedom degrees ($GL = N - 1 = 10 - 1 = 9$) is 1.833.

Therefore, the decision limit is given by the equation:

$$L_C = t_C \times s \times \sqrt{1+\frac{1}{N}} \qquad [3]$$

N is the number of blank replicates (10). Therefore:

$$L_C = t_C \times s \times \sqrt{1+\frac{1}{N}} = 1.833 \times 0.868 \times \sqrt{1+\frac{1}{10}} = 1.669$$

The detection limit corresponds to the double of the decision limit, consequently:

$$L_D = 2L_C = 3.338$$

The detection limit is given in terms of concentration by:

$$x_D = 2x_C = \frac{2L_C}{b} \qquad [4]$$

Where b is the rate of the calibration plot [1]. Therefore:

$$x_D = \frac{2L_C}{b} = \frac{2 \times 3.338}{4\text{E-}06} = 8.346 \cdot 10^{-7}$$

Consequently, the detection limit of **3** at a concentration of $C_6 = 10^{-4}$ M and tryptamine in water by fluorescence measures, corresponds to $8.346 \cdot 10^{-7}$ **M.**

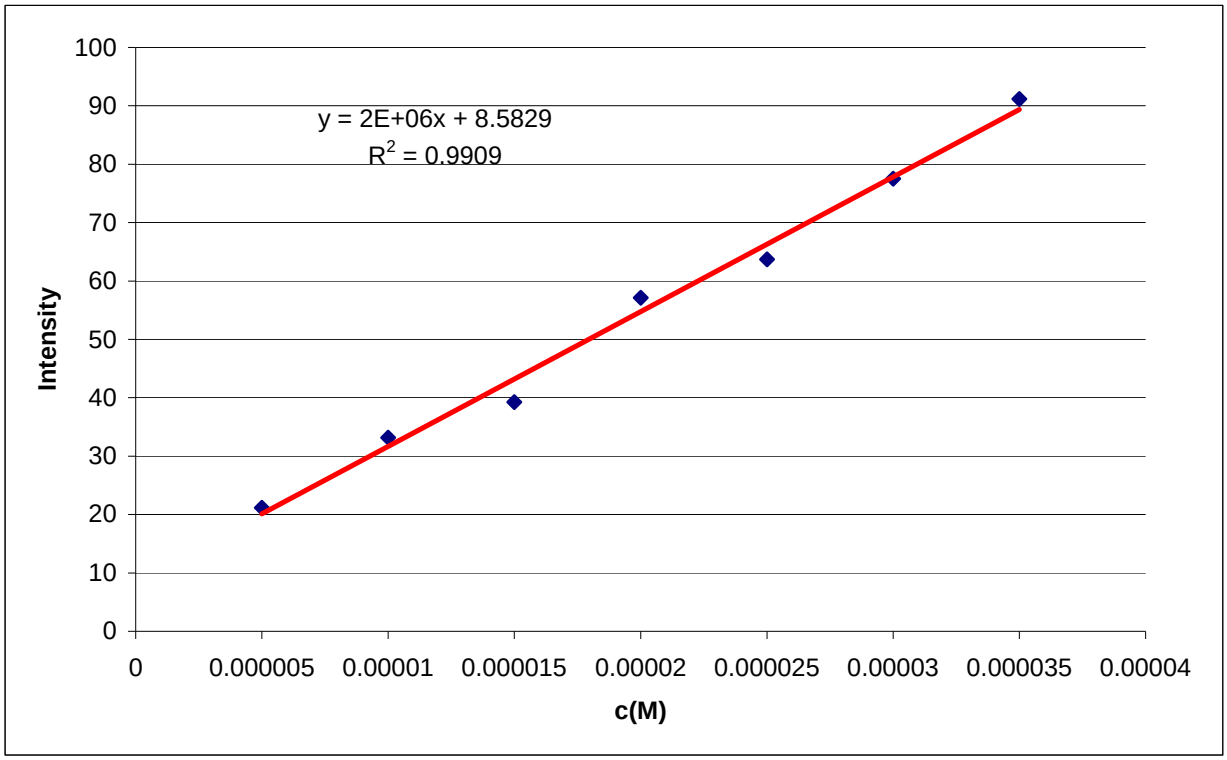
Calculation of the Detection Limit of 6 in DMSO + ephedrine in water

The detection limit was performed by using the two solvents previously used for the rest of essays, DMSO for the solution of **3** and water for the solution of ephedrine. The method used for the calculations is the blank variability as described in the *Handbook of Chemometrics and Qualimetrics*[5]. For this purpose, first a calibration plot is performed, then it is validated and then, in the same conditions, several blank replicates are measured. In this case, the solution of **3** gives a signal without the presence of the analyte, therefore this signal is taken as the blank; 10 replicates of the blank were measured. A calibration with 5 measures and 10 replicates for the blank were taken from a 10^{-4} M solution of **3**:

[5] D. L. Massart, B. G. M. Vandeginste, L. M. C. Buydens, S. De Jong, P. J. Lewi, J. Smeyers-Verbeke, *Handbook of Chemometrics and Qualimetrics: Part A*, Eselvier, Amsterdan, The Netherlands, **1997**, Chapt. 13, 379.

c(M) ephedrine	Intensity
0.000005	21.12969971
0.00001	32.62852097
0.000015	38.99669266
0.00002	57.23438263
0.000025	66.03394318
0.00003	74.98625946
0.000035	90.00195313

With these values the calibration curve was drawn:



The adjusted equation by least squares is obtained:

$$y = 2E + 06x - 8.949 \qquad [1]$$

Then, 10 replicates for the blank were taken. The measured blank emission values, from a solution of **6**, and the values of the concentration calculated from equation [1] are detailed.

replicates	Intensity
1	9.50595284
2	9.18525028
3	9.21925926
4	9.46058846
5	9.01752853
6	8.84387016
7	9.60622311
8	8.66969585
9	8.9730463
10	8.56337929

The mean ($\bar{x}$) and the standard deviation (s) from the fluorescence data are:

$$\bar{x} \pm s = (9.154 \pm 0.098) \quad [2]$$

For a probability level of 5% (α=β=0.05), the value of t_C=t_D from bibliography,[1] for 9 freedom degrees ($GL = N - 1 = 10 - 1 = 9$) is 1.833.

Therefore, the decision limit is given by the equation:

$$L_C = t_C \times s \times \sqrt{1 + \frac{1}{N}} \quad [3]$$

N is the number of blank replicates (10). Therefore:

$$L_C = t_C \times s \times \sqrt{1 + \frac{1}{N}} = 1.833 \times 0.098 \times \sqrt{1 + \frac{1}{10}} = 0.189$$

The detection limit corresponds to the double of the decision limit, consequently:

$$L_D = 2L_C = 0.379$$

The detection limit is given in terms of concentration by:

$$x_D = 2x_C = \frac{2L_C}{b} \quad [4]$$

Where b is the rate of the calibration plot [1]. Therefore:

$$x_D = \frac{2L_C}{b} = \frac{2 \times 0.189}{2\text{E-}06} = 1.898 \cdot 10^{-7}$$

Consequently, the detection limit of **3** at a concentration of $C_6 = 10^{-4}$ M and ephedrine in water by fluorescence measures, corresponds to $1.898 \cdot 10^{-7}$ **M.**

Calculation of the Detection Limit of 3 in DMSO + pseudoephedrine in water

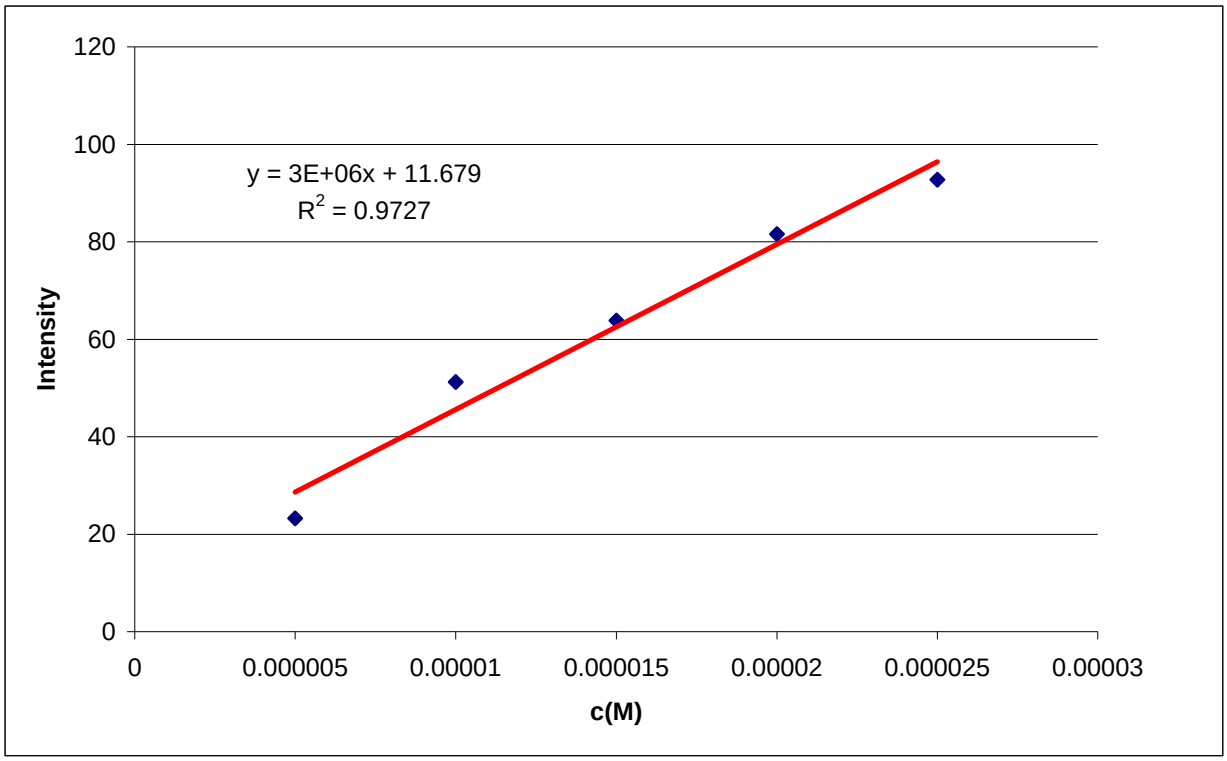
The detection limit was performed by using the two solvents previously used for the rest of essays, DMSO for the solution of **3** and water for the solution of pseudoephedrine. The method used for the calculations is the blank variability as described in the *Handbook of Chemometrics and Qualimetrics*[6]. For this purpose, first a calibration

[6] D. L. Massart, B. G. M. Vandeginste, L. M. C. Buydens, S. De Jong, P. J. Lewi, J. Smeyers-Verbeke, *Handbook of Chemometrics and Qualimetrics: Part A*, Eselvier, Amsterdan, The Netherlands, **1997**, Chapt. 13, 379.

plot is performed, then it is validated and then, in the same conditions, several blank replicates are measured. In this case, the solution of **3** gives a signal without the presence of the analyte, therefore this signal is taken as the blank; 10 replicates of the blank were measured. A calibration with 5 measures and 10 replicates for the blank were taken from a 10^{-4} M solution of **3**:

c(M) pseudoephedrine	Intensity
0.000005	23.22055244
0.00001	51.2039032
0.000015	63.81771088
0.00002	81.60835266
0.000025	92.75402832

With these values the calibration curve was drawn:



The adjusted equation by least squares is obtained:

$$y = 3E + 06x - 11.679 \qquad [1]$$

Then, 10 replicates for the blank were taken. The measured blank emission values, from a solution of **3**, and the values of the concentration calculated from equation [1] are detailed.

replicates	Intensity
1	23.9698448
2	23.6268387
3	23.1652355
4	23.2915516
5	21.8179951
6	22.090292
7	22.4975357
8	22.0392723
9	21.1957302
10	21.2636433

The mean ($\bar{x}$) and the standard deviation (s) from the fluorescence data are:

$$\bar{x} \pm s = (22.495 \pm 0.976) \quad [2]$$

For a probability level of 5% (α=β=0.05), the value of t_C=t_D from bibliography,[1] for 9 freedom degrees ($GL = N - 1 = 10 - 1 = 9$) is 1.833.

Therefore, the decision limit is given by the equation:

$$L_C = t_C \times s \times \sqrt{1 + \frac{1}{N}} \quad [3]$$

N is the number of blank replicates (10). Therefore:

$$L_C = t_C \times s \times \sqrt{1 + \frac{1}{N}} = 1.833 \times 0.976 \times \sqrt{1 + \frac{1}{10}} = 1.876$$

The detection limit corresponds to the double of the decision limit, consequently:

$$L_D = 2L_C = 3.752$$

The detection limit is given in terms of concentration by:

$$x_D = 2x_C = \frac{2L_C}{b} \quad [4]$$

Where b is the rate of the calibration plot [1]. Therefore:

$$x_D = \frac{2L_C}{b} = \frac{2 \times 1.876}{3\text{E-}06} = 1.250 \cdot 10^{-6}$$

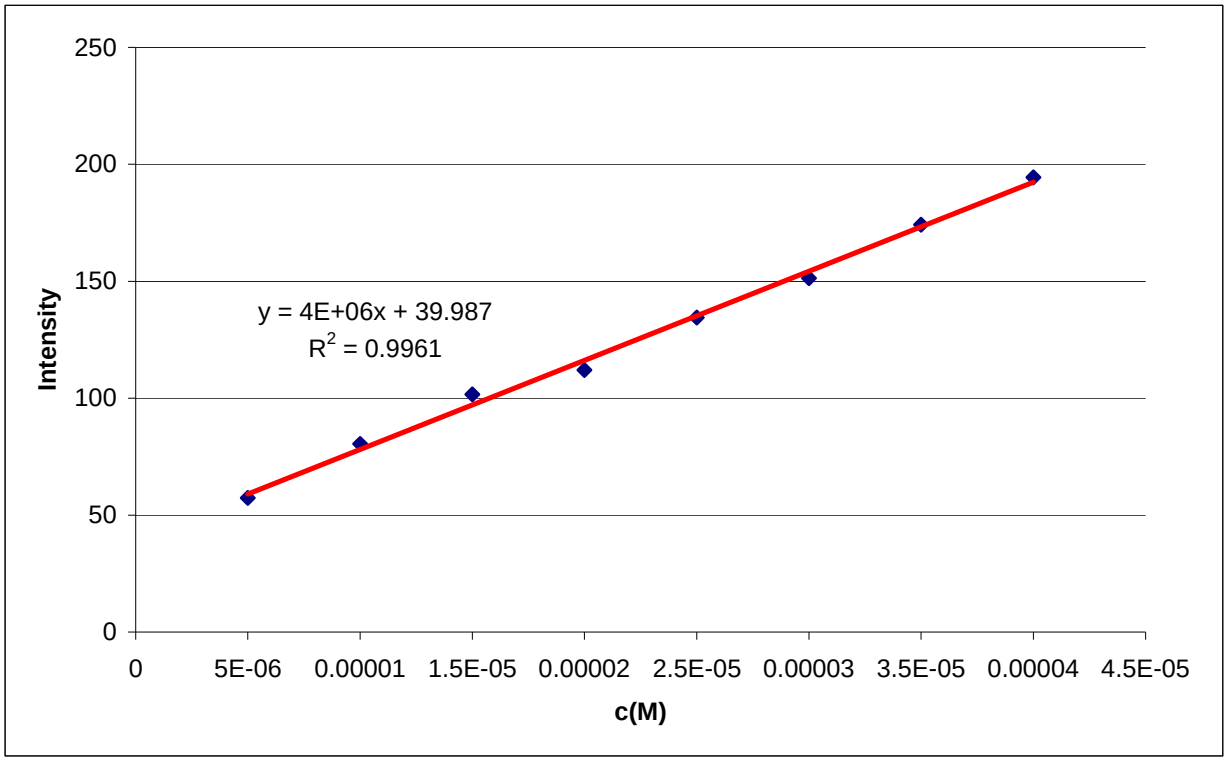
Consequently, the detection limit of **3** at a concentration of C_6 = 10^{-4} M and pseudoephedrine in water by fluorescence measures, corresponds to $1.250 \cdot 10^{-6}$ **M.**

Calculation of the Detection Limit of 3 in DMSO + MDA in water

The detection limit was performed by using two solvents, DMSO for the solution of **3** and water for the solution of MDA. The method used for the calculations is the blank variability as described in the *Handbook of Chemometrics and Qualimetrics*[7]. For this purpose, first a calibration plot is performed, then it is validated and then, in the same conditions, several blank replicates are measured. In this case, the solution of **3** gives a signal without the presence of the analyte, therefore this signal is taken as the blank; 10 replicates of the blank were measured. A calibration with 5 measures and 10 replicates for the blank were taken from a 10^{-4} M solution of **3**:

c(M) MDA	Intensity
0.000005	57.30054474
0.00001	80.40509033
0.000015	101.607933
0.00002	112.0621109
0.000025	134.505722
0.00003	151.3577881
0.000035	174.2579803
0.00004	194.4051056

With these values the calibration curve was drawn:



The adjusted equation by least squares is obtained:

$$y = 4E + 06x - 39.987 \qquad [1]$$

[7] D. L. Massart, B. G. M. Vandeginste, L. M. C. Buydens, S. De Jong, P. J. Lewi, J. Smeyers-Verbeke, *Handbook of Chemometrics and Qualimetrics: Part A*, Eselvier, Amsterdan, The Netherlands, **1997**, Chapt. 13, 379.

Then, 10 replicates for the blank were taken. The measured blank emission values, from a solution of **3**, and the values of the concentration calculated from equation [1] are detailed.

replicates	Intensity
1	40.8143501
2	40.6610832
3	40.7860375
4	40.2789421
5	38.936245
6	39.9844322
7	38.4784584
8	38.7967682
9	38.4589005
10	38.4399033

The mean ($\bar{x}$) and the standard deviation (s) from the fluorescence data are:

$$\bar{x} \pm s = (39.563 \pm 1.032) \quad [2]$$

For a probability level of 5% (α=β=0.05), the value of t_C=t_D from bibliography,[1] for 9 freedom degrees ($GL = N - 1 = 10 - 1 = 9$) is 1.833.

Therefore, the decision limit is given by the equation:

$$L_C = t_C \times s \times \sqrt{1 + \frac{1}{N}} \quad [3]$$

N is the number of blank replicates (10). Therefore:

$$L_C = t_C \times s \times \sqrt{1 + \frac{1}{N}} = 1.833 \times 1.032 \times \sqrt{1 + \frac{1}{10}} = 1.985$$

The detection limit corresponds to the double of the decision limit, consequently:

$$L_D = 2L_C = 3.969$$

The detection limit is given in terms of concentration by:

$$x_D = 2x_C = \frac{2L_C}{b} \quad [4]$$

Where b is the rate of the calibration plot [1]. Therefore:

$$x_D = \frac{2L_C}{b} = \frac{2 \times 1.985}{4\text{E-}06} = 9.924 \cdot 10^{-7}$$

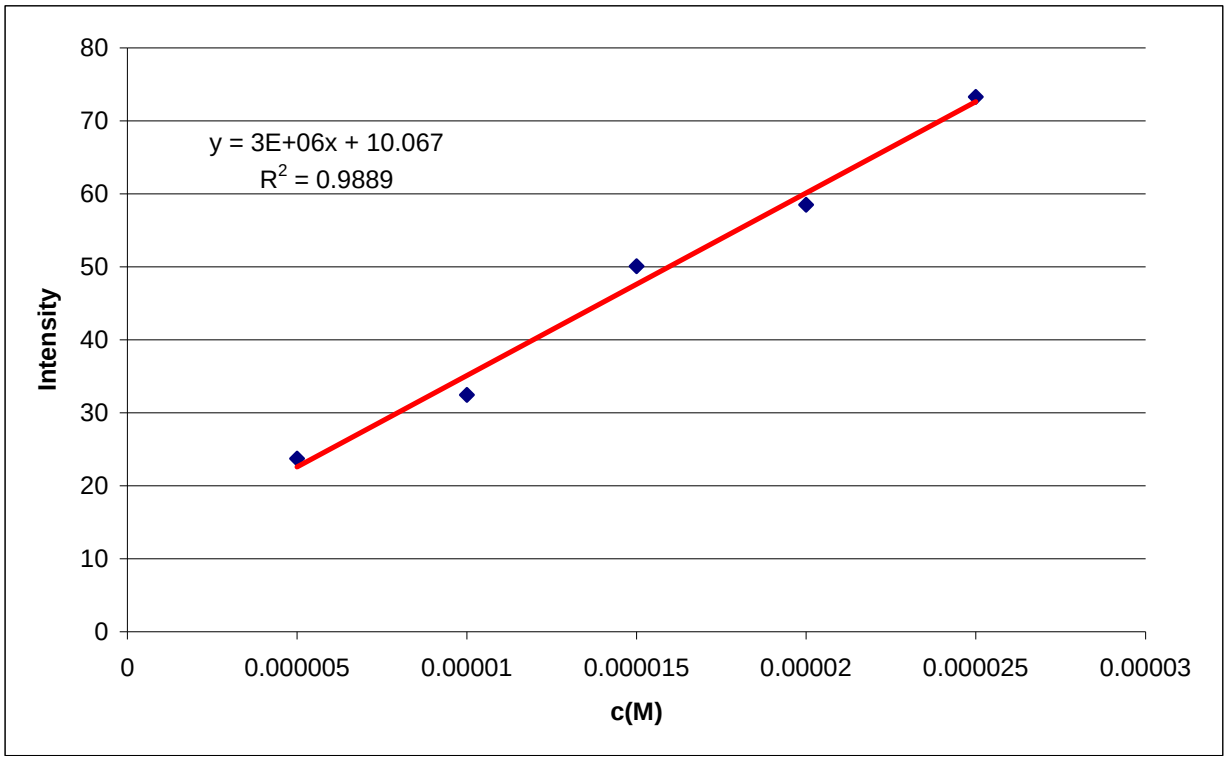
Consequently, the detection limit of **3** at a concentration of $C_6 = 10^{-4}$ M and MDA in water by fluorescence measures, corresponds to $9.924 \cdot 10^{-7}$ **M.**

Calculation of the Detection Limit of 3 in DMSO + MDMA in water

The detection limit was performed by using two solvents, DMSO for the solution of **3** and water for the solution of MDMA. The method used for the calculations is the blank variability as described in the *Handbook of Chemometrics and Qualimetrics*[8]. For this purpose, first a calibration plot is performed, then it is validated and then, in the same conditions, several blank replicates are measured. In this case, the solution of **6** gives a signal without the presence of the analyte, therefore this signal is taken as the blank; 10 replicates of the blank were measured. A calibration with 5 measures and 10 replicates for the blank were taken from a 10^{-4} M solution of **3**:

c(M) MDMA	Intensidad
0.000005	23.72634697
0.00001	32.45100784
0.000015	50.0962677
0.00002	58.49233627
0.000025	73.27461243

With these values the calibration curve was drawn:



The adjusted equation by least squares is obtained:

$$y = 3E + 06x - 10.067 \qquad [1]$$

[8] D. L. Massart, B. G. M. Vandeginste, L. M. C. Buydens, S. De Jong, P. J. Lewi, J. Smeyers-Verbeke, *Handbook of Chemometrics and Qualimetrics: Part A*, Eselvier, Amsterdan, The Netherlands, **1997**, Chapt. 13, 379.

Then, 10 replicates for the blank were taken. The measured blank emission values, from a solution of **3**, and the values of the concentration calculated from equation [1] are detailed.

replicates	Intensity
1	9.16627693
2	9.10811329
3	9.30694199
4	8.99962807
5	9.14923191
6	9.27844524
7	9.02067471
8	9.12172985
9	9.19433117
10	9.2023859

The mean ($\bar{x}$) and the standard deviation (s) from the fluorescence data are:

$$\bar{x} \pm s = (9.154 \pm 0.098) \quad [2]$$

For a probability level of 5% (α=β=0.05), the value of t_C=t_D from bibliography,[1] for 9 freedom degrees ($GL = N - 1 = 10 - 1 = 9$) is 1.833.

Therefore, the decision limit is given by the equation:

$$L_C = t_C \times s \times \sqrt{1 + \frac{1}{N}} \quad [3]$$

N is the number of blank replicates (10). Therefore:

$$L_C = t_C \times s \times \sqrt{1 + \frac{1}{N}} = 1.833 \times 0.098 \times \sqrt{1 + \frac{1}{10}} = 0.189$$

The detection limit corresponds to the double of the decision limit, consequently: $L_D = 2L_C = 0.379$

The detection limit is given in terms of concentration by:

$$x_D = 2x_C = \frac{2L_C}{b} \quad [4]$$

Where b is the rate of the calibration plot [1]. Therefore:

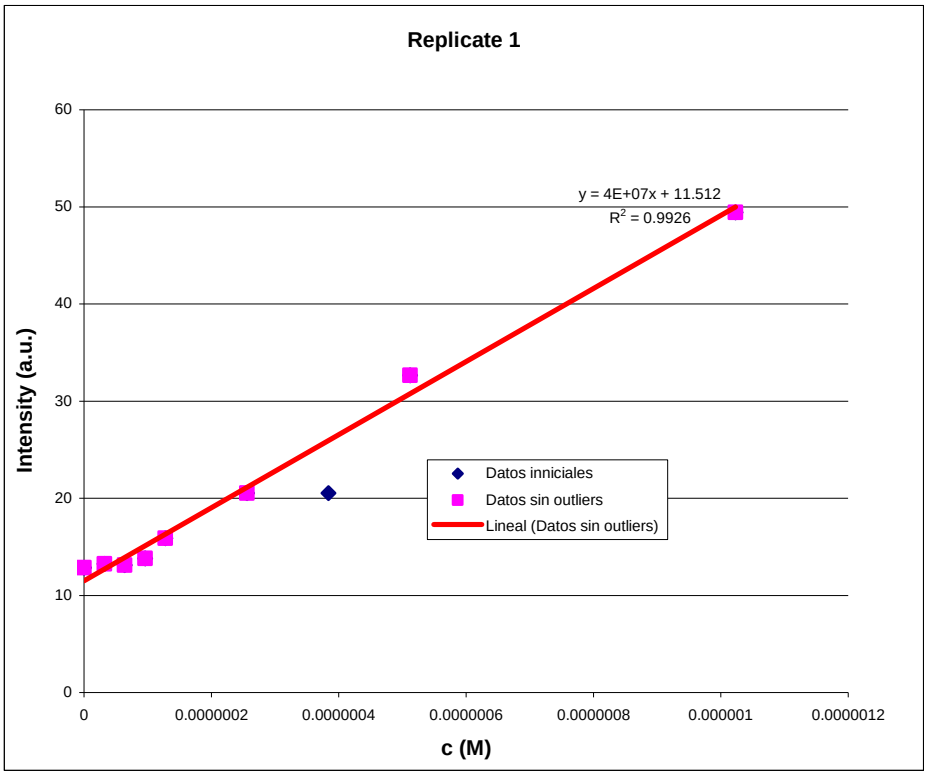
$$x_D = \frac{2L_C}{b} = \frac{2 \times 0.189}{3\text{E-}06} = 1.265 \cdot 10^{-7}$$

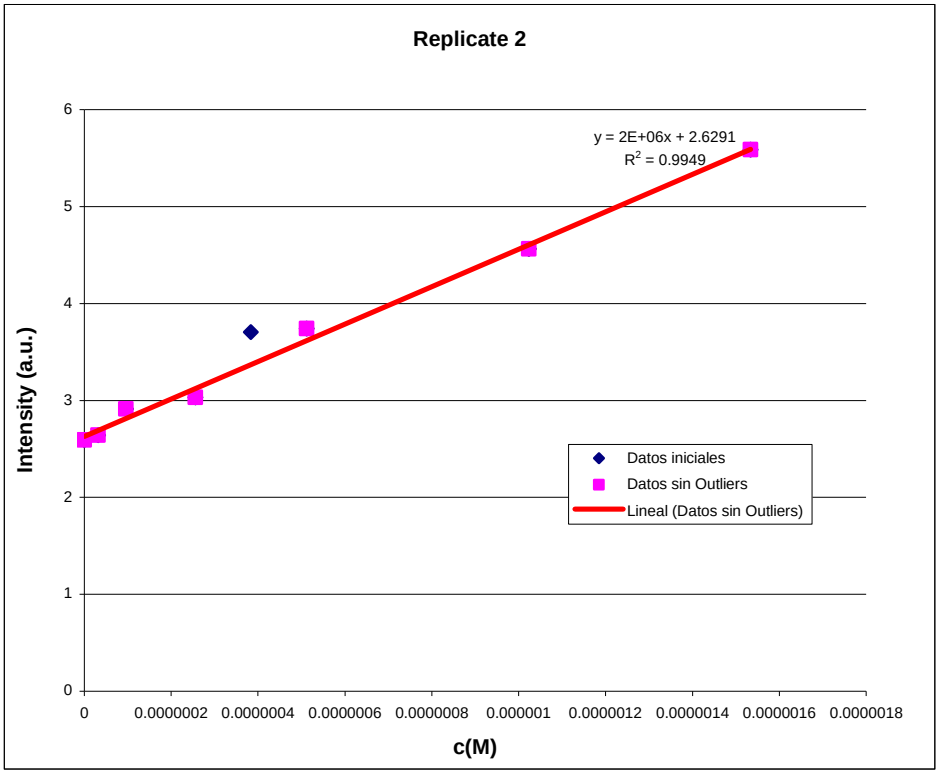
Consequently, the detection limit of **3** at a concentration of $C_6 = 10^{-4}$ M and MDMA in water by fluorescence measures, corresponds to $1.265 \cdot 10^{-7}$ **M.**

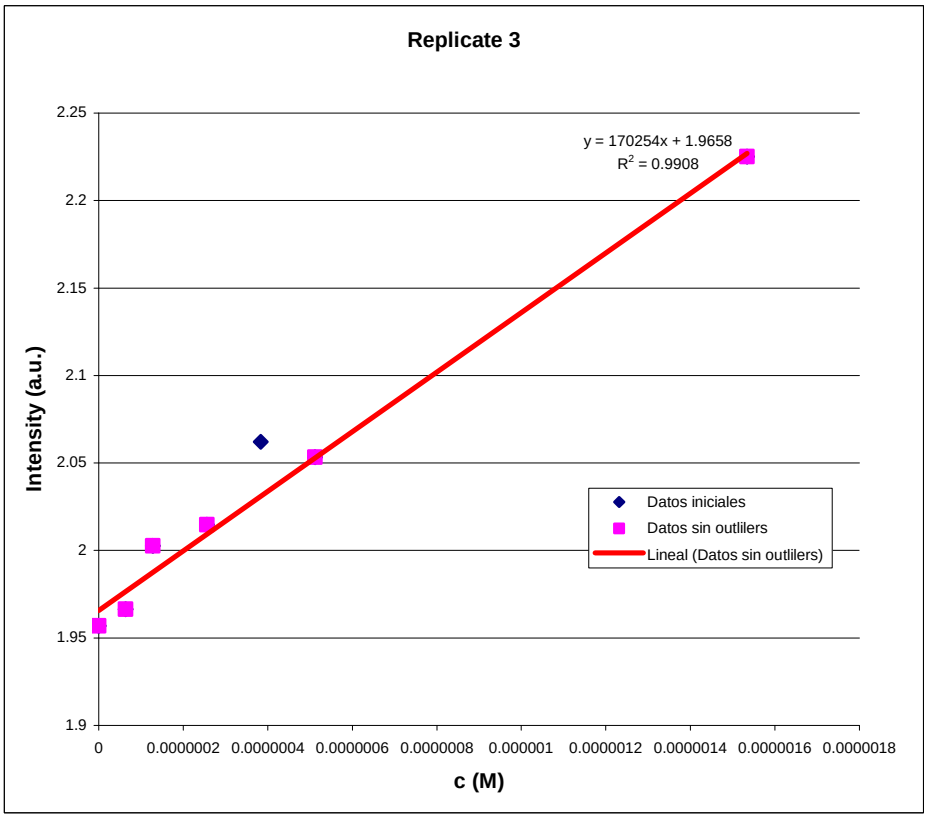
Validation of the Detection Limit of 3 in DMSO + ephedrine in water.

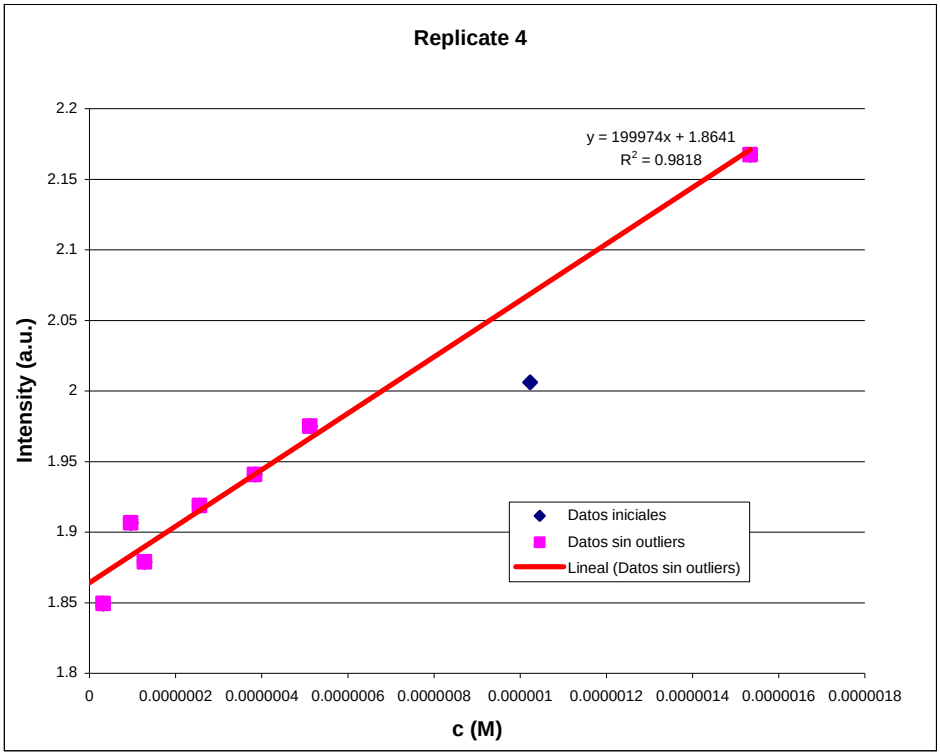
This time the detection limit was calculated by performing a calibration plot built with concentrations of analyte close to the expected detection limit. The calibration plot was built by using a concentration of probe **3** of 5 x 10^{-5} M in DMSO, by addition of growing amounts of ephedrine in water, from a concentration 3.2 x 10^{-8} M to 1.5 x 10^{-6} M. With the obtained results, first a least median squares (LMS) regression was used. The criterion is to minimize the median of squares of the differences between the experimental and the calculated values. LMS regression has the advantage of being able to detect anomalous points, the ‘‘outliers’’, and look for a linear range if at least 50% of the data are aligned. This last property, called ‘‘exact fit’’, is of great interest for the determination of the linear range of analytical processes; habitual deviations from the line, whether at high or low concentrations, are easily detected by the LMS regression once it has been determined that the points are aligned. The strategy followed consisted of two steps. In the first, the LMS regression was used to detect anomalous points, taking a point as ‘‘outlier’’ if the absolute value of the standardized residual is greater than 2.5. In the second step, the anomalous points detected were eliminated and a regression based on the ordinary least-squared (OLS) criterion was carried out, in order to obtain the optimal precision and accuracy of both slope and intercept. Then the detection limit was calculated for the conditions in which $\alpha = \beta = 0.5$. For elimination of outliers the R.2.7.0 program was used.

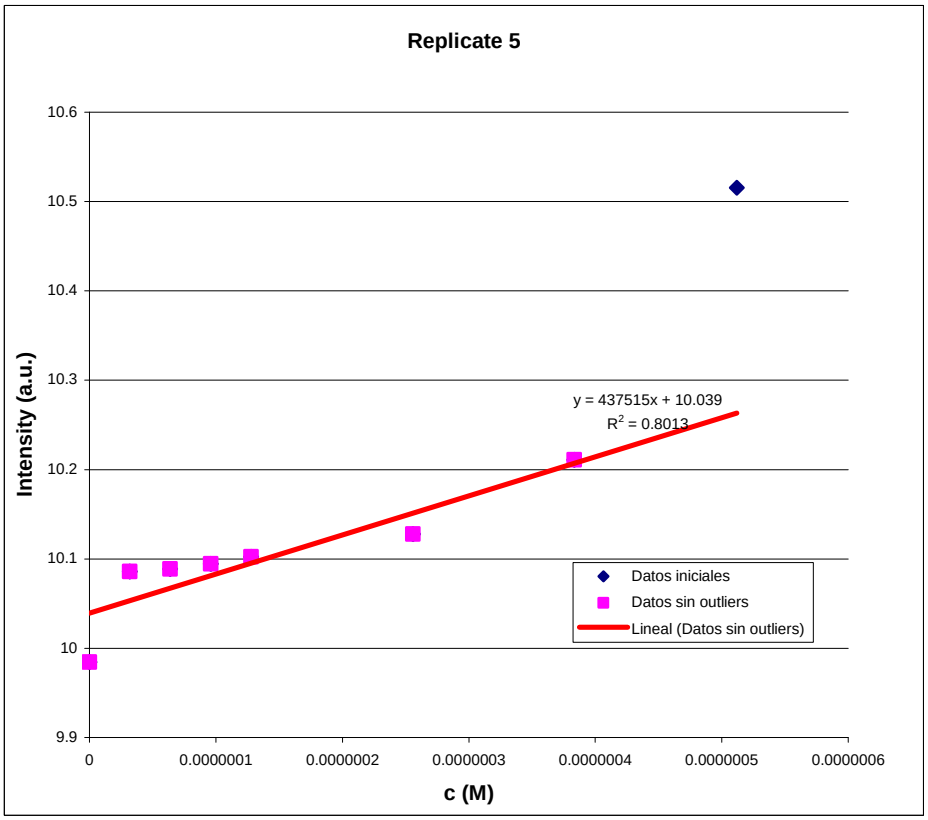
5 Calibration plots were performed.




Replicate 2
y = 2E+06x + 2.6291
R2 = 0.9949
Intensity (a.u.)
c(M)
Datos iniciales
Datos sin Outliers
Lineal (Datos sin Outliers)
0
1
2
3
4
5
6
0
0.0000002
0.0000004
0.0000006
0.0000008
0.000001
0.0000012
0.0000014
0.0000016
0.0000018


Replicate 3
y = 170254x + 1.9658
R2 = 0.9908
Intensity (a.u.)
c (M)
Datos iniciales
Datos sin outlilers
Lineal (Datos sin outlilers)
1.9
1.95
2
2.05
2.1
2.15
2.2
2.25
0
0.0000002
0.0000004
0.0000006
0.0000008
0.000001
0.0000012
0.0000014
0.0000016
0.0000018





The obtained detection limits were:

	Detection Limit
Replicate 1	3.91E-07
Replicate 2	5.86E-07
Replicate 3	5.86E-07
Replicate 4	5.86E-07
Replicate 5	4.46E-07
	$(5.19\pm0.89)\cdot10^{-7}$

The obtained detection limit is of the same order of the detection limit for ephedrine calculated by the blank variability, therefore validating the previous measurements.