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New Spectrofluorimetric Method for Determining Serotonin: Application to Human Urine

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Abstract

In this present paper, we investigate the development of simple, rapid, accurate, reproducible and sensitive methods for the determination of serotonin (5-HT) in urine. For this purpose stationary fluorescence was used as method. With regard to the analysis of serotonin, the liquid-liquid extraction (LLE) solid phase extraction (SPE) and standard addition procedures were used. Several physicochemical factors affecting the sensitivity of the fluorescence intensity of serotonin were optimized, including the system of solvent (organic, micellar), the pH and the salts. The study of the analytical performances of the method led to very low limits of detection (LOD) varying between 0.1 and 3 ng/mL and to limits of quantification (LOQ) ranging between 0.4 and 10 ng/mL. This confirms the sensitivity of the method. Thus the low values of standard deviations (DRS) (between 0.3 and 6.6%) testify the good reproducibility of the measurements with satisfactory covering rate (89 to 111%). Accordingly, our results show that the spectrofluorimetric method is simple, fast and sensitive and can be applied to the routine analysis and does not require expensive equipment nor tiresome chemical pretreatments.

Keywords: serotonin, effects of solvent, method of analysis of fluorescence direct, analysis of urine

1. Introduction

Serotonin (5-HT) is a monoamine neurotransmitter that is primarily found in the gastrointestinal tract and central nervous system. Serotonin was originally discovered by Vittorio Erspamer in 1935 (Qin, 2013). It was isolated, chemically identified as 5-hydroxytryptamine (5-HT), and named by Rapport and al. in 1948 (Rapport, Green, & Page, 1948; Best, Frederik Nijhout, & Reed, 2010; De Ponti, 2004; Berumen, Rodrıguez, Miledi, & Garcıa-Alcocer, 2012). Initially identified as a vasoconstrictor substance in blood serum, approximately 80-90 percent of the human body's total serotonin is located in the enterochromaffin cells in the gut, where it is used to regulate intestinal movements. The remainder is synthesized in serotonergic neurons in the Central Nervous System and in blood platelets. It is also found in fungi and plants.

In the CNS, serotonin is involved in many physiological processes such as: regulation of the sleep/wake cycle, control of body temperature, blood pressure, food intake and sexual and maternal behavior. In the periphery, 5-HT is mainly stored in platelets. Then it participates in the regulation of hemostasis, heart functions or of the gastrointestinal motility. The involvement of serotonin system in such a diversity of functions suggests that the synthesis of 5-HT is subject to a fine regulation. The rate-limiting enzyme for the biosynthesis of serotonin is tryptophan hydroxylase (TPH) (Karan, Timurkaan & Aydin, 2011). Serotonin is widely distributed in brain, and plays significant role in brain function together with other neurotransmitters.

The most common laboratory test for the determination of a serotonin-secreting carcinoid tumor is the measurement of urinary 5-hydroxyindoleacetic acid (5-HIAA), a major serotonin metabolite (Meijer, Kema, Volmer, Willemse, & De Vries, 2000; Kema, Meijer, Meiborg, Ooms, Willemse & De Vries (2001); Kema, De Vries, Schellings, Postmus, & Muskiet, 1992; Zlatuse, Milton, & Steven, 2003; Lee, Cheng, Yeh, & Liou, 2000). However, in the case of tumors producing only small amounts of serotonin, the predictive values of an increased urinary 5-HIAA is low (Meijer, Kema, Volmer, Willemse, & De Vries, 2000). In addition, the concentration of 5-HIAA in urine may be incresead by consumption of foods rich in 5-HT (Nassar, Varshney, Getek, & Cheng, 2001; Patel, Arundell, Parker, & Yeoman, 2005). It has been shown that platelet serotonin is the most sensitive indole marker for the diagnostic of caracinoid tumors, especially those with low serotonin production (Meijer & al., 2000; Kema et al., 2001; Kema et al., 1992). There are many approaches to quantify levels of the 5-HT in human urine including voltammetry (Michael & Wightman, 1999; Baur, Kristensen, May, Wiedemann, & Wightman, 1988), capillary electrophoresis (C.E Lunte, Martin & S.M Lunte, 2000; Wallingford & Ewing, 1987), chemical luminescence (Marklova, Makovickova, & Krakorova, 2000), electron capture (CDE) or mass spectrometric (MS) detection (Milner, Hall, Davis, Brissie, & Robinson, 1998; Eap et al., 1998; Fontanille, Jourdil, Villier, & Bessard, 1997; Shah, Jan, Khan, & Durrani, 2012; Esrafili, Yamini, & Shariati, 2007), enzyme immunoassay, radio immunoassay (Chauveau, Fert, Morel, & Delagee, 1991; Engbaek & Voldby, 1982; Filik, Avan, & Aydar, 2014; Wei, et al., 2014; Zhu, Steiner, Munn, Daws, & Heewlett, 2007). All the recent assays were based either on high-performance liquid chromatography (HPLC) with fluorimetric or UV detection (Ravindra, 2014; Agrawal et al., 2013; Akerman, Jolkokonen, Huttunen, & Penttila, 1998), or on gas chromatography (GC) with nitrogen phosphorus detector (NPD).

Several techniques have been reported for the determination of serotonin concentration in different biological samples, including HPLC with electrochemical determination of serotonin in Rabbit's brain (fresh) (LOD = 0.2 μg/mL) (Z. Wang, Liang, Y. Wang & Luo, 2003), from human platelet-rich plasma (LOQ = 2.3-4.6 pg/mL) (Tekes, 2008), in biological tissue (LOD = 11.86-33.62 ng/mL) (Patel, Arundell, Parker, Yeoman & O'Hane, 2005), in the pineal gland of juvenile golden hamsters, (Harumi, Akutsu, & Matsushima, 1996), in chicken brain tissue (LD = 5 pg per injection) (Qu, Moons & Vandesande, 1997), and rat-brain (0.94 pg). HPLC with fluorimetric detection was used for the determination of 5-HT in urine (LOD = 7.0 nmol/L), and in rat brain (LOD = 5.0 nmol/L) (Yoshitake et al., 2004; Ishida et al., 1998).

Capillary electrophoresis or gas chromatography with electron capture (ECD), or nitrogen phosphorus detection (NPD) or mass spectrometric (MS) detection were used for the determination of serotonin in human plasma (Peterson, Lee & Graves, 2004).

As 5-HT may be got in the urine of person sick, we decided to develop this analytical method, known as to be quick, easy, cheap, sensitive, selective, ease-of-use, low cost and labor minimal solvent usage. We were also interested in investigating quantitative determination of serotonin in urine.

In this present work, we investigated the fluorescence spectral properties of 5-HT in various organic solvents and aqueous media (different pH, various salt and micellar environments). A spectrofluorimetric method was developed and used for the direct determination of serotonin in human urine using liquid-liquid extraction, solid phase extraction and standard addition procedures.

2. Experimental

2.1 Reagents and Solvents

All chemicals were of analytical grade. 5-hydroxytryptamine (5-HT) (99%, w/w), was purchased from Alpha Aesar (France), sodium hydroxide (97%, w/w) and hydrochloric acid (36%, m/m) used were obtained from Sigma Aldrich (Taufkirchen, Germany). Salts as K_2SO_4 (99.6% m/m), $Ca_3P_4O_8$ (90% m/m), NaH_2PO_4 (98% m/m), were also obtained from Sigma Aldrich. Sodium dodecyl sulfate (SDS, 98% m/m), cetyltrimethylammonium chloride (CTAC, 25% in water), cetyltrimethylammonium hydroxide (CTAOH, 10% in water) and Brij-700 pellets (99 % m/m, n = 100) from Sigma-Aldrich and were used in amounts greater than the critical micelle concentration (cmc). Solvents such as methanol, ethanol, acetonitrile, dichloromethane, ethyl acetate, dimethylsulfoxide (DMSO) and cyclohexane were obtained from Merck (Darmstadt, Germany). Distilled water was used to prepare aqueous solutions of these substances.

2.2 Apparatus

For our measurements, we used a spectrofluorimeter Perkin Elmer LS-55 model connected to a microcomputer and driven by the FL WinLab software. A parallelepiped quartz cuvette (1 cm optical path, inside volume 3.5 ml) with five smooth surfaces was used for the analytical measurements. Weighing was carried out using a precision

balance of accuracy 0.1 mg from Sartorius AG Gottingen (Type BA 110S-OF1). The dilutions were performed using brand micropipettes Gilson, France.

2.3 Procedure

2.3.1 Stock Solutions Preparation

Stock solutions of 5-HT (0.02 M) were freshly prepared by dissolving the compound in distilled water. Serial dilutions were performed to obtain working solutions. Stock solutions of HCl (0.5 M) and NaOH (0.1 M) were prepared with distilled water and used for serial dilutions.

Standard solutions were: K_2SO_4 (1 M), $Ca_3P_4O_8$ (1 M) and NaH_2PO_4 (1 M). Stock solutions of surfactants (SDS; CTAC; CTAOH; Brij-700, 0.02 M) were prepared in 50 mL volumetric flasks with distilled water and used for serials dilution. Freshly prepared solutions were protected against sunlight by covering with aluminium foil and preserved by storing in a refrigerator at 6°C for further use.

2.3.2 Samples Preparation

Extraction of 5-HT in urine was made on samples freshly collected and diluted with distilled water. The samples were used within a day to avoid denaturation.

Urine samples to be analyzed were subjected to preliminary step of deproteinization by using concentrated perchloric acid (6 N). Interfering proteins and other large amino-molecules were precipitated by concentrated acid.

2.3.2 Direct Spiking Procedures

5-HT was extracted from fortified tap water and from urine samples by using liquid-liquid extraction method with ethyl acetate, chloroform and methanol used as solvents.

A volume of 9 mL of tap water or urine was diluted to one-tenth and fortified with 1 mL solution of 5-HT (10^{-6}M) in a flask. This solution was then introduced into a separatory funnel of 250 mL in which 10 mL of extraction solvent was added. After 10 min of manual agitation, the organic phase was isolated while the aqueous fraction was subjected to a second extraction. After a third extraction of the aqueous phase, all organic phases were combined, then evaporated to dryness. The residue was then recovered with 10 mL of distilled water. Then 500µL of this later solution was put in a vial and adjusted with distilled water to 10 mL. Measuring the fluorescence intensity of the extract allows us to assess the percent recovery of 5-HT in water. The percent recovery was evaluated by the average of the respective recovery rates of various solutions of 5-HT at different concentrations (20, 50 and 100 ng/mL). Our results showed that the most important recovery rate was that obtained when the pH of the aqueous solution was 12. The pH of the actual sample of serotonin was adjusted to 12 with phosphate sodium buffer; this allowed saturating the aqueous solution.

2.3.3 Solid Phase Extraction Procedure (SPE)

A C18 cartridge was used to extract the serotonin in the urine samples. The cartridge was conditioned by percolating a volume of 5 mL of the extracting solvent (acetonitrile, methanol, etc.). Then we passed 10 mL of fortified urine sample at three concentrations (20, 40 and 60 ng / mL). Final elution was done with 5 mL of the same extractant.

2.3.4 Standard Addition Method

We first performed the calibration curve using tap water as solvent in order to assess the applicability of the method used, after we used human urine samples. For this purpose, we have strengthened our samples with concentrations ranging 22.5 to 150.4 ng/ml of 5-HT, then we performed in 8 vials, progressive additions of solution of 5-HT, taking the first addition as the blank.

3. Results and Discussion

Firstly, we investigated the fluorescence spectral properties of 5-HT in organic solvents and various aqueous media (different pH, various salt and organized media) and we optimized the analytical conditions; secondly, we therefore developed an analytical procedure for the direct spectrofluorimetric determination of 5-HT in urine by direct spiking, solid-phase extraction and standard addition procedures.

3.1 Preliminary Study

We observed fluorescence stability of the molecule of serotonin (5-HT) in all the solvents studied. The excitation and emission spectra were recorded at 25°C. The shapes of the spectra were identical in all the solvents used (two excitation peaks and one emission peak). For example in water, the excitation spectrum has two peaks

located at 223 nm and 275 nm respectively, while the emission one was located at 340 nm (Fig.1).

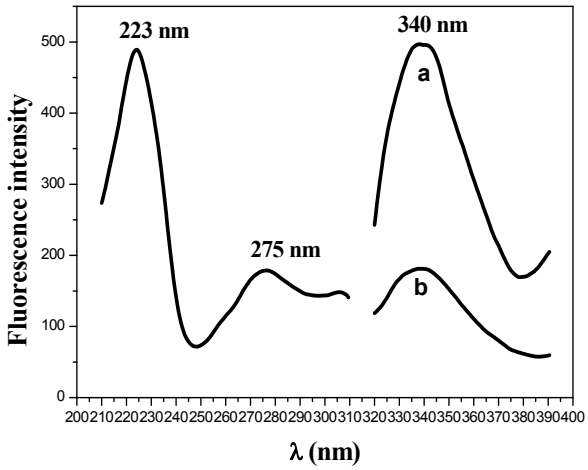

Figure 1. Fluorescence excitation and emission spectra of 5-HT (10^{-7} M) in water at 298 K
a) emission spectra at λ_{ex} = 223nm b) emission spectra at λ_{ex} = 275nm

3.2 Optimization of the analytical Parameter

3.2.1 pH Effect in Micellar Media and Media Aqueous

Studies have shown that the fluorescence signal of organic compounds is very sensitive to the pH of the medium (Coly & Aaron, 2009). Thus, to a prepared solution of serotonin 10^{-7} M, we added some known amount of acid or base corresponding to a theoretical value of pH. Subsequently, we plotted the curve representing the variation of fluorescence intensity versus theoretical pH. Even in the absence of commercial buffer solutions, this curve (Fig. 2) gave us an idea about the dependence of the fluorescence signal of 5-HT with the pH. It was observed a maximum signal in the pH range between 2 and 10. Thus, this result indicated that it was possible to work in neutral aqueous solution, during the analysis of real samples.

The pH is also a factor affecting the fluorescence of the analytes in organized media. Therefore, it is important to know its impact on the fluorescence emission of 5-HT in the different micellar solutions used (CTAC, CTAOH, SDS and Brij 700).

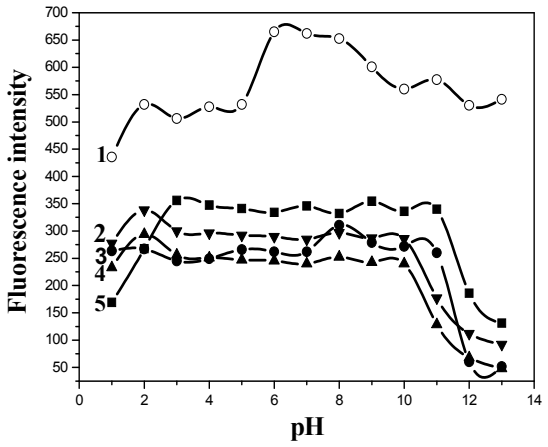

Figure 2. pH effect on the fluorescence intensity of 5-HT(10^{-7} M) in different media (10^{-2} M).
1: Brij-700, 2: CTAH, 3: CTAC, 4: water, 5: SDS at 298 K. (λ_{ex} = 275 nm ; λ_{em} = 340 nm).

The results obtained in different solvent systems (Fig.2) showing the curves of variation of the fluorescence intensity as a function of pH were similar in most of the solvents used. Indeed, with the exception of Brij 700 where there are large intensities in all pH ranges, the studied solvents exhibit maximum fluorescence range for pH values situated between 2 and 10. However, for all these solvent systems, the previous study revealed no significant information on the stability of the fluorescence signal of 5-HT. In fact, the points belonging to these curves were recorded at relatively short times. Thus, for more information on the stability of fluorescence, the kinetic study was then developed.

3.2.2 Kinetic Study of 5-HT in Micellar Media

The kinetics of fluorescence of 5-HT in micellar solution was carried out for 30 minutes (Fig.3). We noted that the fluorescence signal of 5-HT was relatively stable in water and in the micellar aqueous solution of SDS at pH 7; However, for Brij 700 for which a decrease in the signal versus time was observed after about four minutes, the addition of ether oxide has improved the stability of the signal with the same initial intensity.

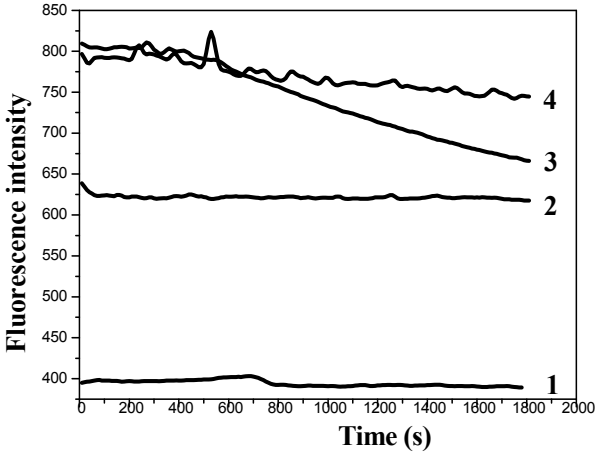

Figure 3. Evolution of the fluorescence intensity of 5-HT (5.10^{-7} M) over time in different media at 298 K. (1: water; 2: SDS (8 mM); 3: Brij 700 (8 mM); 4: Brij-700 + ether oxide (8 mM) at pH = 7.

3.2.3 Solvents Effect

Here we propose to examine the effects of various solvents (water, methanol, ethanol, acetonitrile, dimethyl sulfoxide, cyclohexane, ethyl acetate, dichloromethane) (Fig.4) and micelles (SDS, Brij 700, CTAC, CTAOH) (Fig.5) on the fluorescence signal of 5-HT. The results are summarized in table 1.

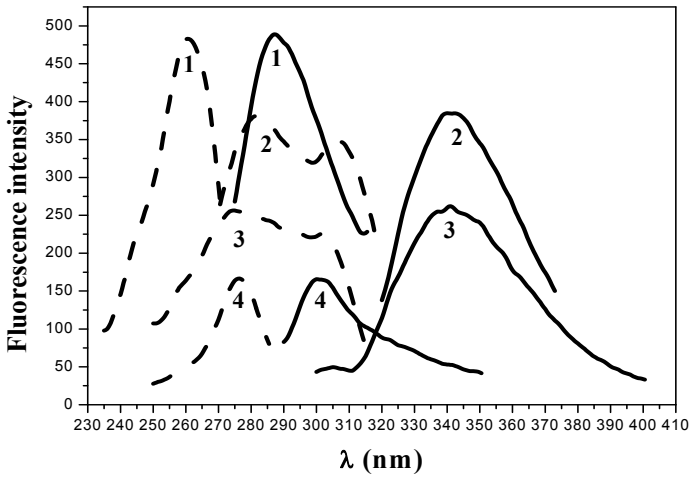

Figure 4. Fluorescence excitation and emission spectra of 5-HT in different organic solvents
(1: Acetonitrile, 2: Dimethylsulfoxyde, 3: water, 4: Cyclcohexane)

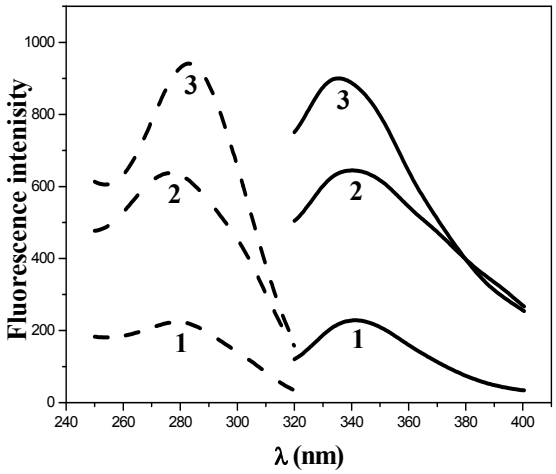

Figure 5. Fluorescence excitation and emission spectra of 5-HT (10^{-7} M)
in different micellar media. 1: water; 2: SDS; 3: Brij 700 (λ_{ex} = 275 nm; λ_{em} = 340 nm).

For this study, we prepared a stock solution of 5-HT (10μM) in distilled water. Then, we introduced 50 μL of this initial solution in a volumetric flask of 5 mL before adjusting each time with the desired solvent. The proportion of water in the organic solvent was 1%. All the results are summarized in Table 1.

Table 1. Fluorescence Spectroscopic properties of 5-HT (10^{-7}M) in different solvents studied.

Solvent	$\lambda_{ex}/\lambda_{em}$ (nm) [(a)]	I_F [(b)]	ε [(c)]
Cyclohexane	276/300	1.28	2.02
Ethyl acetate	260/306	4.00	6.02
Dichloromethane	318/367	1.00	8.93
Acetonitrile	260/287	3.78	37.5
Dimethylsulfoxyde	282/340	2.69	46.7
Ethanol	275/340	2.00	24.5
Methanol	274/338	2.03	32.7
water	275/340	1.65	78.4
Brij 700 (pH = 7)	283/336	6.85	-
CTAC (pH = 7)	276/341	1.76	-
CTAOH (pH = 7)	276/341	1.99	-
SDS (pH = 7)	276/340	4.98	-

[(a)] excitation (λ_{ex}) et emission (λ_{em}) wavelengths.

[(b)] Relative fluorescence intensity, normalized relative to the fluorescence intensity in dichloromethane for 5-HT

[(c)] Dielectric constant

If in non polar organic solvents we have in general a single excitation peak, in polar solvents we have two or one peak and a shoulder. However in micellar medium, there was a single excitation peak. In any case, we have only one emission peak in all studied solvents. If the excitation spectra show a slight bathochromic shift from non polar solvent, to polar solvent, this displacement is much more pronounced in fluorescence. This might be due to the large change in the polarity of the excited state of the molecule relatively to the fundamental state, which would imply a significant distortion of the molecule in the excited state (Cisse et al., 2014). For micelles, we observed a significant exaltation of the fluorescence signal compared to water; this is consistent with previous studies (Coly & Aaron, 2009).

The analysis of Table 1 shows that Brij 700, SDS, and ethyl acetate have presented favorable conditions for analytical application with very high relative emission intensities (Fig.5).

3.2.4 Effect of Binary Mixture (Water / Methanol And Water / Ethanol) on Fluorescence Spectra of Serotonin

The extraction can be carried out in solvents as methanol or ethanol, it is important to maximize the percentage of water/alcohol which gives the best fluorescence signal of serotonin in this binary medium. For this, we recorded the fluorescence spectrum for each mixture: water/methanol and water/ethanol, v/v. The figure 6 shows that the proportion in volume of water / methanol (1: 1, v/v) and water / ethanol (70:30, v/v) are the more suitable for the analysis of serotonin in these binary mixtures, at least taking into account the emission intensities.

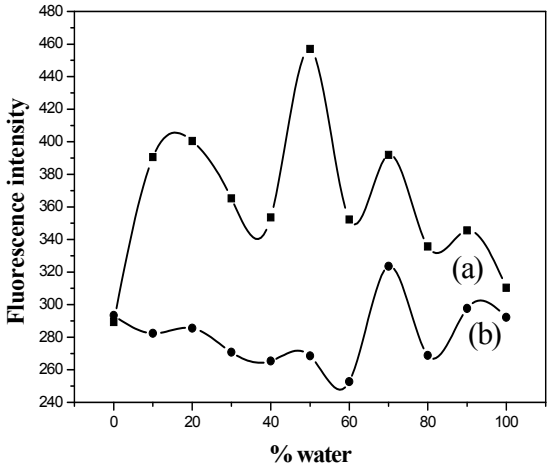

Figure 6. Effect of the variation of water percentage of different mixtures on the fluorescence signal of 5-HT (5.10^{-7} M) Water/methanol (a) and water/ethanol (b).

3.2.5 Salts Effect

We found it necessary to examine the effects of biogenic amines and salts due to their strong presence in urine. Thus, we studied the effect of various inorganic salts on the fluorescence intensity of 5-HT in aqueous medium.

Indeed, the heavy metal target organs are varied: the metal ions bind to red blood cells (Pb, Cd, CH_3Hg). Many metals also accumulate in the liver and kidney (highly vascularized organs), while lead accumulates preferably in teeth and bones. Similarly, soluble metals in lipids as tetraethyl lead or methylmercury can enter the central nervous system. In this way, children are more vulnerable and are often subject to severe lead poisoning because their blood-brain barrier is not fully developed. Moreover, thanks to their lipid solubility by passive diffusion, cadmium, lead, nickel, methyl mercury, are able to cross the placenta and can accumulate (FAO-OMS, 2003).

3.2.6. Added Foreign Interference Studies of Species

To establish their possible interference on the determination of 5-HT, we were interested to two biogenic amines (histamine and putrescine) as well as inorganic ions (Na^+, Cl^-, K^+, Mg^{2+}, etc.) which may be present in body fluids. For each interfering species, we determined the fluorescence spectra of 5-HT (10^{-7}M) in water by successively adding biogenic amine concentrations (10^{-4} M to 4.10^{-3} M) and various salts concentrations (10^{-4} M to 0.3 M). The results obtained allow us to determine each time the percentage of variation of the transmitting signal of 5-HT. We observed no change of the shape of the fluorescence spectra when increasing the concentration of the amine or salt; however, a small decrease in the fluorescence intensity was observed. This result shows that there was no specific interaction between 5-HT and interfering species (Agrawal et al., 2013; Akerman et al., 1998).

We have grouped in table 3 the values of the tolerance limit and the concentration of critical salts of each interfering species. The boundary is defined as the limiting concentration for which the percentage of variation

of the fluorescence signal $\mathbf{\Delta F\ (\%) = [(F_0 - F)/\ F_0]\ x\ 100}$, did not exceed ± 5%. In this expression, F_0 and F indicate, respectively, the fluorescence signal of serotonin in the absence and in the presence of interfering species. Among the studied ions, Mg^{2+} ion produces the greatest effect of interference with tolerance limit of $1.5.10^{-5}$ M. The radical change in the intensity observed with a maximum resulting from a certain concentration of salt (5.10^{-3}M, NaCl for example) (Fig.8), corresponding probably to the presence of an aggregation mechanism of 5-HT therewith. This particular salt concentration is defined as the concentration of critical salt (CSC) (Adenier & Aaron, 1999).

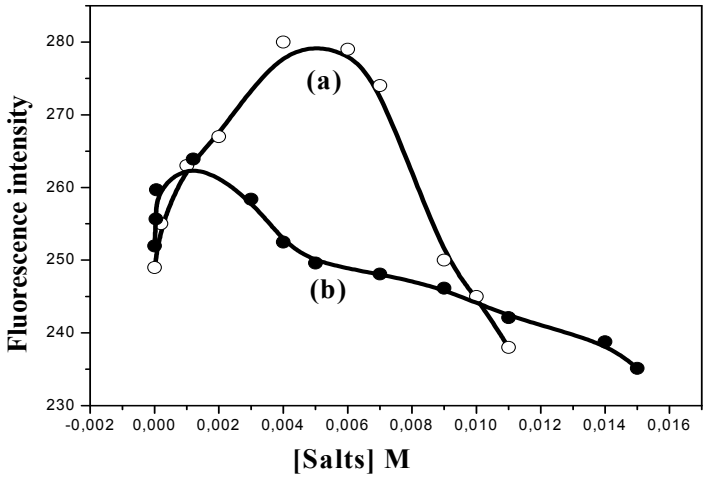

Figure 7. Salt effect on 5-HT (10^{-7} M) fluorescence in water at 298 K
(a) NaCl and (b) KNO_3

Critical salt concentration (CSC) was determined at the maximum point of respective curves

Table 2. Study of foreign species interference with 5-HT[a].

Foreign species	λ_{ex} / λ_{em} [a] (nm)	CSC[b](M)	Tolerance limit [c](M)
Na^+Cl^-	277/339	0.00476	0.011
K^+NO^{3-}	274/347	0.0012	0.011
$Mg^{2+}SO_4^{2-}$	277/342	0.00365	$1.5x10^{-5}$
$Ca^{2+}(PO4)_2^{3-}$	294/362	0.14546	0.0013
Putrescine	275/340	-	0.002
Histamine	275/340	-	0.001

[a] excitation and emission wavelengths;

[b] critical salt concentration (M);

[c] See text for definition

4. Analytical Performance

To assess the analytical value of the proposed method, we have established a calibration curve of serotonin in different extraction solvents as Brij-700 (pH = 7), ethyl acetate, methanol and chloroform; we also performed the calibration in water in order to compare the experimental results. We have chosen these solvents because they had a very important emission intensity with 5-HT. In all solvents we have obtained linear correlations between the fluorescence intensity and the concentration of 5-HT (Fig.8 and Table 3), with correlation coefficients (r^2) of 0.999 close to the unity indicating the accuracy of our measurements. From linear calibration curves obtained, we determined the analytical parameters (Table 3). The study of the analytical performance of the method leads to limits of detection (LOD) were very low ranging between 0.1 and 3 ng/mL and the limit of quantification (LOQ) of between 0.4 and 10 ng/mL; this confirms the sensitivity of the method. This table also shows that the limits of detection (LOD) are 3 to 30 times lower in the Brij 700 than in organic solvents and water. Also, the linear dynamic range is much greater with the Brij700.

Table 3. Spectrofluorimetric analytical figures of merit for the determination of 5-HT in organic solvents, in micellar solution and aqueous media

Medium	LDR [a] (ng/mL)	LOD [b] (ng/mL)	LOQ [c] (ng/mL)	RSD [d](%)	r^{2} [e]
Water (pH = 2)	15-200	0.8	3.0	2.2	0.999
Brij-700 (pH =7)	10-300	0.1	0.4	1.3	0.998
Ethyl acetate	10-220	0.3	1.0	0.3	0.999
Methanol	15-210	3.0	10.0	3.1	0.999
Chloroforme	15-210	2.0	6.0	6.6	0.999

[a] Linear dynamic range.

[b] Limit of detection, defined as the amount of analyte giving a signal-to-noise ratio of 3.

[c] Limit of detection, defined as the amount of analyte giving a signal-to-noise ratio of 10.

[d] Mid-range relative standard deviation (n = 6-8).

[e] Correlation coefficients.

5. Analytical Applications

To verify the applicability of the method to real samples, we first determined serotonin in tap water after a partitioning extraction procedure (RMS). The pH of the tap water as that of the urine sample was adjusted to 12 before the liquid-liquid extraction process. The recovery percentages obtained were between 89.0 and 96.5 (Table 4). These results therefore showed some observed losses with the use of this method. To improve the extraction procedure, we then applied the procedure of extraction solid phase (SPE). In this case, the extraction of serotonin present in the urine showed recovery rates between 95.0 and 101.0% for the water / methanol mixture (1/1, v / v) and between 93.0 and 98.0% for chloroform (Table 5). Thus, for the latter method, extraction in water-methanol mixture seems more suitable. To verify the applicability of the method to real samples, we first determined serotonin in tap water after a partitioning extraction procedure (RMS). The pH of the tap water as that of the urine sample was adjusted to 12 before the liquid-liquid extraction process. The recovery percentages obtained were between 89.0 and 96.5 (Table 4). These results therefore showed some observed losses with the use of this method. To improve the extraction procedure, we then applied the procedure of extraction solid phase (SPE). In this case, the extraction of serotonin present in the urine showed recovery rates situated between 95.0 and 101.0% for the water/methanol mixture (1/1, v/v) and between 93.0 and 98.0% for chloroform (Table 5). Thus, for the latter method, extraction in water-methanol mixture seems more suitable.

To better quantitative analysis of 5-HT in the urine, we used the standard addition curve to assess the degree of interference caused by the presence of other compounds (Diaw et al., 2014; Mbaye et al., 2011). In both cases, the slopes of the calibration lines and normal addition standards obtained are very close. This parallelism between the two curves showed that, the involvement of the interfering phenomena was little sensitive. This reflects that the matrix effects are very negligible in our samples. Satisfactory recovery rates between 100.0 and 111.8% were found (Table 6). These values are consistent with international standards of validation of the analytical methods (Diaw et al, 2014). To improve quantitative analysis of 5-HT in the urine, we used the standard addition curve to assess the degree of interference caused by the presence of other compounds (Diaw et al., 2014; Mbaye et al., 2011). In both cases, the slopes of the calibration lines and normal addition standards obtained are very close. This parallelism between the two curves showed that, the involvement of the interfering phenomena was little sensitive. This reflects that the matrix effects are very negligible in our samples. Satisfactory recovery rates situated between 100.0 and 111.8% were found (Table 6). These values are consistent with international standards of validation of the analytical methods (Diaw et al., (2014).

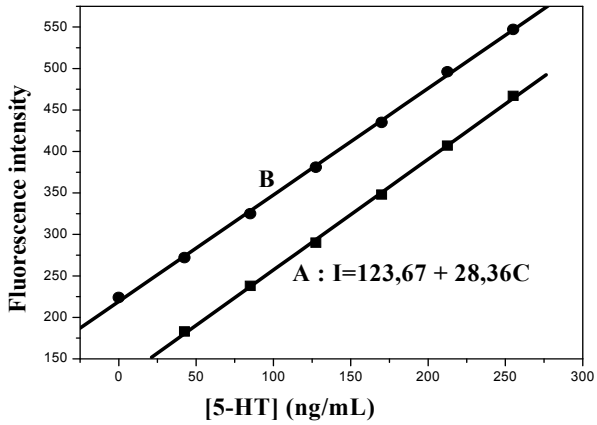

Figure 8: Straight calibration curve:
A): Calibration curve of serotonin in Methanol;
B): standard addition curve of the sample in urine (λ_{ex} = 223 nm, λ_{em} = 340 nm);

Table 4. Evaluation of recovery values in urine obtained by direct spiking procedure

Direct spiking procedure			
Added (ng/mL)	Found (ng/mL)	Recovery (%)	Mean Recovery (%)
20	19.2	96.5	93.2±4.6
50	44.5	89.0	
100	94.6	94.6	

Table 5. Evaluation of recovery values in urine by solid-phase extraction procedure

Solid-phase extraction procedure (SPE)				
Solvents	Added (ng/mL)	Found (ng/mL)	Recovery (%)	Mean Recovery (%)
Methanol/Water (1/1,v/v)	20	19.0	95.0	98.2±0.6
	40	39.2	98.7	
	60	60.6	101.0	
Chloroforme	20	18.6	93.0	95.5±1.2
	40	39.2	98.0	
	60	57.3	95.5	

Table 6. Evaluation of recovery values in urine by standard addition

Standard addition procedure (SPE)			
Added (ng/mL)	Found (ng/mL)	Recovery (%)	Mean Recovery (%)
0	21.3	-	105±0.4
22.5	47.6	108.7	
43.9	67.6	103.7	
65.0	89.3	103.5	
86.3	109.7	101.9	
107.6	128.9	100.0	
128.8	167.8	111.8	
150.4	184.2	107.3	

6. Conclusion

We have developed in this work a simple, sensitive, accurate and inexpensive method for the determination of serotonin in urine samples. We have demonstrated the utility of the spectrofluorimetric analysis with the optimization of the fluorescence of 5-HT in the binary mixture water/methanol (1:1, v/v). The Analytical actual values confirmed the good sensitivity and accuracy of the method used for the determination of serotonin. We also showed the applicability of the method used for the determination of 5-HT in the urine samples at the level of $ng.ml^{-1}$, with satisfactory recovery values. The reproducibility and simplicity of the method for direct determination by fluorescence of 5-HT are satisfactory for routine analysis; in addition, this method of analysis by spectrofluorimetric ways does not require expensive equipment or tedious chemical pretreatments.

Therefore, the results obtained in this study show the interest of the analytical spectrofluorimetric method for the quantitative analysis of serotonin (5-hydroxytryptamine) in urine samples. In our laboratory LPA, we plan to combine spectrofluorimetric detection with flow injection system to improve the speed of analysis for routine determination of biogenic amines in physiological fluids.

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