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## Manual or automated measuring of antipsychotics' chemical oxygen demand

Sarah A.P. Pereira<sup>a</sup>, Susana P.F. Costa<sup>a</sup>, Edite Cunha<sup>a</sup>, Marieta L.C. Passos<sup>a,\*</sup>,  
 André R.S.T. Araújo<sup>a,b,\*\*</sup>, M.Lúcia M.F.S. Saraiva<sup>a,\*</sup>

<sup>a</sup> LAQV, REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, Porto University, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

<sup>b</sup> Unidade de Investigação para o Desenvolvimento do Interior, Instituto Politécnico da Guarda, Av. Dr. Francisco de Sá Carneiro, n° 50, 6300-559 Guarda, Portugal



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### ABSTRACT

Antipsychotic (AP) drugs are becoming accumulated in terrestrial and aqueous resources due to their actual consumption. Thus, the search of methods for assessing the contamination load of these drugs is mandatory. The COD is a key parameter used for monitoring water quality upon the assessment of the effect of polluting agents on the oxygen level.

Thus, the present work aims to assess the chemical oxygen demand (COD) levels of several typical and atypical antipsychotic drugs in order to obtain structure-activity relationships. It was implemented the titrimetric method with potassium dichromate as oxidant and a digestion step of 2 h, followed by the measurement of remained unreduced dichromate by titration. After that, an automated sequential injection analysis (SIA) method was, also, used aiming to overcome some drawbacks of the titrimetric method.

The results obtained showed a relationship between the chemical structures of antipsychotic drugs and their COD values, where the presence of aromatic rings and oxidable groups give higher COD values.

It was obtained a good compliance between the results of the reference batch procedure and the SIA system, and the APs were clustered in two groups, with the values ratio between the methodologies, of 2 or 4, in the case of lower or higher COD values, respectively. The SIA methodology is capable of operating as a screening method, in any stage of a synthetic process, being also more environmentally friendly, and cost-effective.

Besides, the studies presented open promising perspectives for the improvement of the effectiveness of pharmaceutical removal from the waste effluents, by assessing COD values.

### 1. Introduction

Surface and groundwater pollution by pharmaceuticals is considered a concern worldwide (Khetan and Collins, 2007).

Although human pharmaceuticals are found at  $\text{ng L}^{-1}$  levels, there are already numerous pharmaceutical compounds at low concentrations in the aquatic environment, (Kostich and Lazorchak, 2008) that due to their persistence exhibit an accumulative pollutant effect and their environmental impact is of concern due to the ecotoxicological effects that these low concentrations can promote in the aquatic environment (Pereira et al., 2017). Additionally, there is an increased use of newly manufactured compounds, for example antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs) that associated with the lack of efficient technologies for wastewater treatment, are causing an increment of the pharmaceutical concentrations in water streams becoming a serious problem in the near future (Yu et al., 2009). So, the

presence and the behavior of these compounds in the aquatic environment need to be addressed in order to improve the quality of environmental health (Fent et al., 2006; Santos et al., 2010). Depaolini et al. evaluated the salbutamol residues in wastewaters and they concluded that the residues values were highly constant over a relatively long time (three months). The authors justify this results maybe because the microbial population and weathering status of samples were stable for the investigation period but also because of the short time spent in the environment (estimated in 7 h) (Depaolini et al., 2016).

The consumption level of the AP drugs has seen a huge increase (171%) during the last years (INFARMED, 2000–2012), but little attention has been given to their environmental fate in comparison to other pharmaceuticals micropollutants (Wilde et al., 2016).

For this reason, the search of methods for assessing the contamination load of these drugs is mandatory.

AP drugs are used in psychiatric patients for treatment of acute

\* Corresponding authors.

\*\* Corresponding author at: LAQV, REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, Porto University, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal.

E-mail addresses: [marietapassos@gmail.com](mailto:marietapassos@gmail.com) (M.L.C. Passos), [andrearaujo@ipg.pt](mailto:andrearaujo@ipg.pt) (A.R.S.T. Araújo), [lsaraiva@ff.up.pt](mailto:lsaraiva@ff.up.pt) (M.L.M.F.S. Saraiva).

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psychotic episodes such as anxiety or in the prevention of relapses in patients with schizophrenia. They may also be administered in patients with other psychotic disorders such as mania, bipolar disorder, and delusional disorders, even when they don't present symptoms of psychosis (Grundmann et al., 2014; Zhang et al., 2007, 2008).

There are two classes of AP drugs: typical or first generation and atypical or second-generation APs. The second generation APs are most effective against both negatives and positives symptoms of psychiatric patients, as in other symptoms like aggressiveness and depressive symptoms. Moreover, second-generation APs can produce less extrapyramidal side effects, tardive dyskinesia, and neuroleptic malignant syndrome when compared to the first generation APs (El-Didamony et al., 2015; Zhang et al., 2007).

All APs have some antagonistic effect on the dopamine  $D_2$  receptors. Regarding the second-generation APs, they also have antagonist activity at some serotonin receptors, especially the receptor 2A (Divac et al., 2014). The differences in the chemical structures of AP drugs play a crucial role in their interactions with neurotransmitter receptors, resulting in their respective neuropharmacological properties (Jafari et al., 2012).

The chemical structures of AP drugs may also present different behavior at the environmental level since all APs have aromatic rings in their chemical structures and the diversity regarding the number, type, and position of substituent groups, determines not only their particular chemical properties but also their environmental fate and behavior (Cvetnic et al., 2017). When industrial, hospital or wastewater treatment plants effluents are analyzed it is difficult to relate observed effects to specific pollutants present in these effluents (Deshpande and Satyanarayan, 2011). However, it was possible to identify the pollutant present in the wastewater using, for example, gas-chromatography-mass spectrometry (GC-MS) and high-performed liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) (Ghoshdastidar et al., 2015).

So, it is important to understand how each pollutant contributes to the environmental fate evaluating each specific pollutant degradability. There is no literature about COD values for these drugs, so it is extremely important to develop a methodology to evaluate the action/disposal capacity of these drugs in the environment.

Chemical oxygen demand (COD), is one of the methods used to determine the quality of water and a key parameter used in environmental pollution monitoring (Zhang et al., 2010). COD is defined as the amount of a specified oxidant that reacts with the sample under controlled conditions. The amount of oxidant consumed is expressed in terms of its oxygen equivalent. The extent of sample oxidation can be affected by digestion time, reagent strength, and sample COD concentration (Eaton et al., 2017). This is also a measure widely used to evaluate the effectiveness of wastewater treatment plants (Aquino et al., 2006)

There are currently technologies that have been studied for the determination of COD and that allow monitoring of the behavior and presence of pharmaceutical compounds in the environment such as: photooxidation (irradiation with ultraviolet (UV) light) (Limonés-Herrero et al., 2014); advanced oxidation processes (oxidation processes which emphasize treating contaminants in water, soil and air, on the presence and reactivity of hydroxyl radicals ( $\text{OH}\cdot$ ) generated in atmosphere and also in water environment or under supercritical conditions of temperature and pressure with or without catalyst, and/or reactive power) (Mendez-Arriaga et al., 2011); electrochemical oxidation or electrodegradation (degradation of pharmaceutical compounds with use of electrodes of different materials) (Santos et al., 2013); ionization (oxidation of pharmaceutical compounds either by a direct reaction with ozone or indirectly with highly reactive radicals (Wilde et al., 2016), among others. However, there are two methods that are most used for determining this parameter: colorimetric and titrimetric (Eaton et al., 2017). The titrimetric method presents some disadvantages such as: incomplete oxidation and mineralization, low sensitivity and

precision, uses large sample volumes, and reagents such as  $\text{Ag}_2\text{SO}_4$ , concentrated  $\text{H}_2\text{SO}_4$  and toxic  $\text{HgSO}_4$ , chemicals not environmentally friendly and causing secondary pollution (Hassan et al., in press; Zhang et al., 2011). Besides that, it involves a time consumption reflux process of 2–4 h which makes it non-applicable for high-throughput screening (Hassan et al., in press). However, this method has advantages compared with the colorimetric. The titrimetric method can be used in samples with high turbidity and residual color while in the colorimetric method this is not possible, especially with a maximum absorption at around 600 nm (Aquino et al., 2006).

Since in the wastewater or in water environment the concentration level of pharmaceuticals are far below the sensitivity of the methods used, there are the possibility to use a pre concentration method, being the solid-phase extraction (SPE) the most frequently used technique for enrichment of trace organic compounds in aqueous samples, as reported by Zgola-Grzeskowiak and Grzeskowiak (2013).

So, in the present work, it was decided to perform the titrimetric method and a new proposed methodology based on sequential injection analysis (SIA), for the determination of a group of drugs that have never been studied before, the antipsychotic (AP) drugs.

It is very important to have data to implement structure-activity relationship (SAR) studies to evaluate their degradability before they enter the environment, as a part of a sustainable development of chemicals in order to clarify the impact of particular structural elements and to guide their modification to reduce their hazardous potential.

It is aimed the evaluation of the COD levels for the chemical oxidation of several AP drugs, both typical and atypical types by performing the reference titrimetric method, and by applying a sequential injection analysis (SIA) the results will be compared and the advantages pointed out.

## 2. Material and methods

### 2.1. Reagents

All solutions were prepared using chemicals of analytical grade with no further purification and water from a MilliQ plus system with specific conductivity of less than  $0.1 \text{ mS cm}^{-1}$ .

All antipsychotic drugs solutions with a concentration of 100 ppm were prepared by using pharmaceutical formulations such as tablets and injectable solutions.

The solutions of chlorpromazine (Largactil IV<sup>®</sup> 50 mg/2 ml injectable solution), levomepromazine (Nozinan<sup>®</sup> 25 mg/ml injection solution) zuclopenthixol (Cisordinol Acutard<sup>®</sup> 50 mg/ml solution for injection), flupenthixol (Fluanxol Retard<sup>®</sup> 100 mg/ml solution for injection), tiapride (Tiapridal<sup>®</sup> 100 mg/2 ml solution for injection), risperidone (Risperidone Sandoz<sup>®</sup> 1 mg/ml oral solution) and haloperidol (Haldol decanoato<sup>®</sup> 50 mg/ml injection solution) were prepared by dilution in water of the commercially available formulations. The solutions of chlorpromazine (powder (P), Sigma-Aldrich), olanzapine (Bluepharma Olanzapine<sup>®</sup> 2.5 mg film-coated tablets), clozapine (Clozapine Generis<sup>®</sup> 25 mg tablets) and cyamemazine (Tercian<sup>®</sup> 100 mg film-coated tablets) were prepared by dissolving the powders in water.

The reagents for performing the COD test using the titrimetric method were: potassium hydrogen phthalate solution equivalent  $5000 \text{ mg L}^{-1} \text{ O}_2$ ; potassium dichromate digestion solution  $0.01667 \text{ mol L}^{-1}$ ; sulfuric acid reagent; ferroin indicator solution and the titrant ferrous ammonium sulfate (FAS)  $0.10 \text{ mol L}^{-1}$ , as described in Standard Methods for the Examination of Water and Wastewater (Eaton et al., 2017).

In SIA system, the carrier solution was ultrapure water. A solution of Ce (IV)  $2 \text{ mol L}^{-1}$  was prepared daily through dissolution of cerium (IV) sulfate in concentrated sulfuric acid to obtain a final concentration of  $3.19 \text{ mol L}^{-1}$ . Glutamic acid/glucose and potassium hydrogen phthalate solutions were used as standard solutions. A stock solution of glutamic acid/ glucose ( $150 \text{ mg L}^{-1}$ ) and intermediate solutions (10;

22.5; 35; 47.5; 60 mg L<sup>-1</sup>) were prepared by dissolving equal quantities of anhydrous glutamic acid and D-(+)-glucose in water. Stock solution of potassium hydrogen phthalate (4250 mg L<sup>-1</sup>) and working solutions (15; 22.5; 45; 60; 90; 120 mg L<sup>-1</sup>) were prepared in water.

## 2.2. Apparatus

To perform the digestion in titration method, it was used a digester Foss tecator® digestion system 12 1009 with Kjeldahl micro tubes with a maximum volume of 100 ml. When occurred digestion, the digester was connected to an extraction system Foss Tecator Scrubber®, in order to retain the gases released during its execution.

The automated COD method was previously reported by Costa and co-authors (Costa et al., 2017). The SIA system consisted of glass syringe of 5 ml volume coupled (Hamilton Bonaduz AG, Switzerland) to a syringe module Bu1S from Crison Instruments S.A. (Allela, Barcelona, Spain) and a 10-port multiposition Cheminert™ selection valve, which was controlled by a computer. Solenoid head-valves allowed the commutation of the syringe either to the manifold or to the carrier. The software used for the instrumental control was developed using Visual Basic and the communication with the instruments was accomplished by means of RS-232C asynchronous protocols, using embedded dynamic libraries. A sequential output of the commands and evaluation of the equipment status was performed through the implementation of a control algorithm based on the use of a set of interdependent timers. The analytical parameters, such as flow rate, flow direction, valve position, stop flow duration, reagents volumes were computer-controlled. The manifold components were connected by a 0.8 mm i.d. PTFE tube, which was also used in the holding and reaction coil (2 and 1 m, respectively). During the assays, the analytical signals were recorded on strip chart recorder (Kipp & Zonen BD 111) or acquired via computer.

A Jasco FP2020 spectrofluorimeter was used on the spectrofluorimetric measurements. The excitation and emission wavelengths were set at 256 and 360 nm, respectively.

The irradiation of the samples was performed with a 15 W Philips TUV 15 W/G15T8 low-pressure mercury lamp at 253.7 nm. A PTFE tubing (90 cm and 0.8 mm i.d.) reactor was looped around the lamp.

## 2.3. Titration method procedure

The titration method is composed of two parts: the sample digestion and the titration of the digested samples.

Before the use, digestion tubes and caps were placed in sulfuric acid 20%.

In the digestion tubes it was added carefully 1.5 ml of the standard dichromate solution, 3.5 ml of the sulfuric acid reagent, and finally 2.5 ml of antipsychotic drug (sample). In the case of blank, it was added to the tubes all of the solutions previously described but instead of the sample, it was added water. The tubes were very well closed with caps and stirred in the vortex until complete mixture. They were then placed in the heating block previously heated to 150 °C for two hours. After

digestion, the tubes were removed from the reactor until room temperature. Thereon, the titration was performed with FAS, after adding 1–2 drops of ferroin indicator.

The final titration was indicated by the change of yellow to blue-green, and finally to reddish brown color.

In some cases, before digestion, there was no remaining oxidant. Thus, it was necessary to perform dilutions of the APs samples with higher COD values.

For each series of samples, it was carried out a blank and a standard control. Each AP drug was evaluate in quadruplicate and a recovery assay were performed.

The COD values obtained by the titrimetric method was calculated using the Eq. (1), (Eaton et al., 2017)

$$COD (mgO_2L^{-1}) = \frac{(A - B) \times M \times 8000}{\text{Sample volume (mL)}} \quad (1)$$

where A and B was the titrant volume used in blank and sample assays, respectively; M was the titrant molarity and 8000 was the oxygen milliequivalents weight x 1000.

For each AP a recovery assay was performed. The value of fortification concentration was 100 mg L<sup>-1</sup>. It was used 0.400 ml of potassium hydrogen phthalate standard solution and it was diluted in a 20 ml flask, with the fortified sample.

The recovery rate was calculated using the Eq. (2):

$$\text{Recovery rate (\%)} = \frac{\text{conc. fortified sample}}{\text{conc. sample} + \text{conc. potassium hydrogen phthalate}} \times 100 \quad (2)$$

For each measurement of the recovery rate, the following values are used: a blank, a standard potassium hydrogen phthalate (mg O<sub>2</sub> L<sup>-1</sup>), one sample of AP (mg O<sub>2</sub> L<sup>-1</sup>) and a fortified AP sample (mg O<sub>2</sub> L<sup>-1</sup>), i.e., AP sample with standard potassium hydrogen phthalate.

## 2.4. Sequential injection procedure

The sequential injection procedure applied for COD determination was performed according to the analytical cycle described by Costa et al. (2017). The SIA system is represented in the next figure (Fig. 1).

The assays were performed at room temperature. Blank assays were performed at the beginning of the working day and repeated 4–5 h later. The blank assays consisted of the replacement of the AP drugs by ultrapure water. Each condition was evaluated in triplicate.

## 3. Results and discussion

In this section, it will be described the implementation of the titration method, the results of the application of the SIA method previously described and the comparison of the COD values obtained with both methods.

So, it was determined the COD values for the following APs: chlorpromazine, clozapine, cyamemazine, flupenthixol, haloperidol,

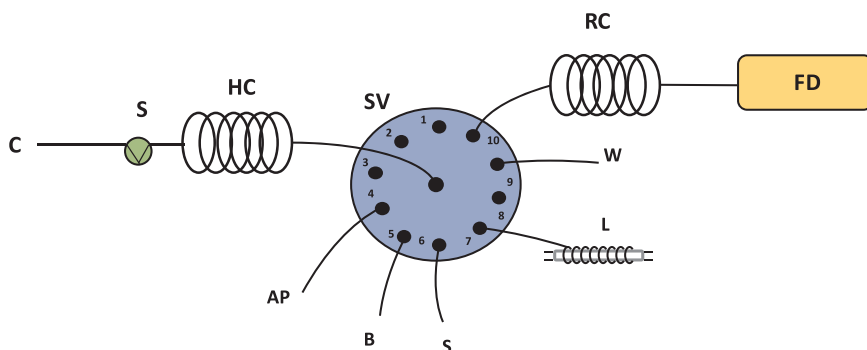


Fig. 1. Schematic representation of the SIA system applied in the determination of COD. C: carrier (ultrapure water); S: syringe (5 ml); HC: holding coil (2 m); SV: selection valve; RC: reaction coil (1 m); FD: fluorimetric detector; AP: antipsychotic drug; B: blank (ultrapure water); S: substrate (Ce(IV)); L: reactor coiled to UV-lamp; W: waste. (Costa et al., 2017).

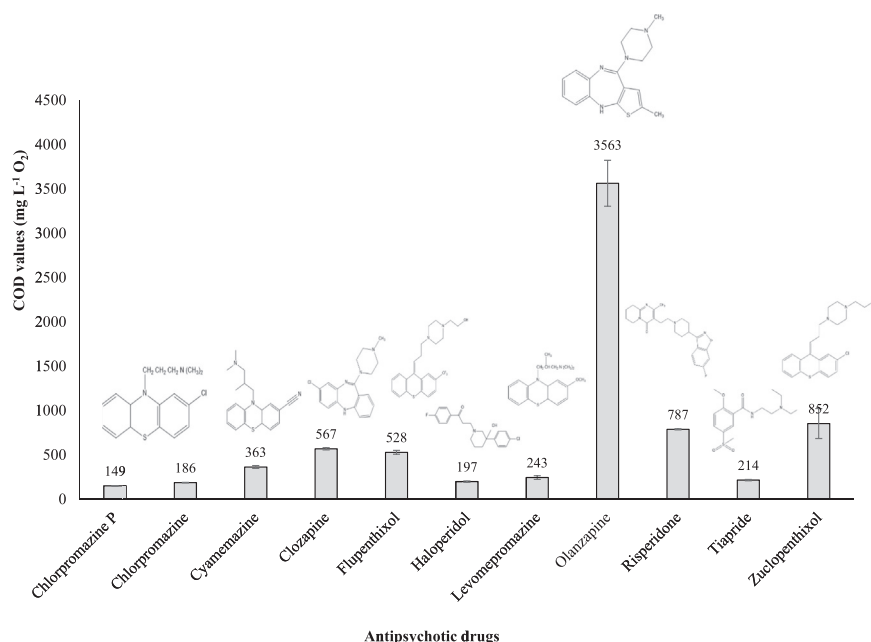


Fig. 2. APs COD values (mg L<sup>-1</sup> O<sub>2</sub>) obtained by the titrimetric method.

levomepromazine, olanzapine, risperidone, zuclophenthixol, and tiapride, whose chemical structures are in Supplementary Material.

### 3.1. Determination of COD of antipsychotic drugs by titration method

Through the analysis of Fig. 2, it was possible to see that the COD values of antipsychotics depend on the compound in question, and so on the different groups present in its structure.

The AP drugs that present higher COD values were olanzapine (3563 mg O<sub>2</sub> L<sup>-1</sup>), zuclophenthixol (852 mg O<sub>2</sub> L<sup>-1</sup>), risperidone (787 mg O<sub>2</sub> L<sup>-1</sup>), clozapine (567 mg O<sub>2</sub> L<sup>-1</sup>) and flupenthixol (528 mg O<sub>2</sub> L<sup>-1</sup>). In the other hand, APs which have a lower COD values were: chlorpromazine powder (P) (149 mg O<sub>2</sub> L<sup>-1</sup>), chlorpromazine (186 mg O<sub>2</sub> L<sup>-1</sup>) and haloperidol (197 mg O<sub>2</sub> L<sup>-1</sup>). Looking to the Eq. (1) from which it is calculated the COD values, it can be seen that the lower amount of titrant used, the greater is the COD, once there is less amount of remaining oxidant in the final of digestion.

The differences in the COD values obtained for the AP drugs can be justified by the chemical structure. APs with higher COD have four or five aromatic rings and more oxidable groups than the other AP drugs. There is evidence that generally, higher molecular mass implies that the compound is less biodegradable and thus has higher COD values (Cvetnic et al., 2017).

Compounds with aromatic rings provide a rigid structure. The previous characteristic combined with the high temperatures of the glass material where the solutions are, justify the good resistance of the compounds even in harsh environments (Gurnule et al., 2014). Therefore, the aromatic rings oxidation takes some time (Morsi et al., 2011) and the removal of COD required the presence of a catalyst for degradation (Chong et al., 2013). In the used method, the silver sulfate was the catalyst for the reaction and this is described as the most effective catalyst for most organic compounds (Boyles, 1997).

As described above, the different obtained COD values can be justified by the presence of more oxidable groups. So, considering only the phenothiazines drugs, such as chlorpromazine, cyamemazine, and levomepromazine, they are oxidized in the functional group in position 2, [chlorpromazine with (-Cl), levomepromazine with (-OCH<sub>3</sub>) and cyamemazine with (-N)]. These AP drugs are also oxidized in the sulfur atom in position 5 (Basavaiah and Swamy, 2001; Kojlo et al., 2000).

On the other hand, looking for all chemical structures, it is possible to observe that the AP drugs that have more atoms groups have higher

COD value. So it seems that the presence of more oxidable groups, increase the COD values. For example, regarding the chemical structure of olanzapine (COD value = 3563 mg O<sub>2</sub> L<sup>-1</sup>) there are seven potential oxidable groups while in chlorpromazine P (COD value = 149 mg O<sub>2</sub> L<sup>-1</sup>) there are only four.

Furthermore, it is possible to verify that the second-generation APs present higher COD values comparing with first-generation APs and it could be justified by the fact that in their chemical structure they present more aromatic rings and oxidable groups than the others.

The Relative Standard Deviation (RSD) values showed the good precision of the method since most of them are less than 5% (Table 1).

AP drugs that have a higher RSD values are zuclophenthixol (19.39%) and levomepromazine (8.59%). These results can be explained due to the presence of very oily excipients in these commercial formulations. Since these compounds are immiscible in water, the aspiration of equivalent quantities of the active substance and the other excipients is more difficult, which results in different COD values between different measurements.

### 3.2. Determination of recovery rate of antipsychotic drugs

Based on the analysis of Table 2, it can be concluded that AP drugs with recovery rates are about 100% were chlorpromazine, haloperidol, and risperidone. Drugs with lower recovery rates, about 50% were

Table 1  
APs COD values as well as the respective Standard Deviation and the RSD obtained by the titrimetric method.

Antipsychotics	Mean	Standard Deviation	RSD (%)
Chlorpromazine P	149	1.60	1.07
Chlorpromazine	186	3.20	1.72
Cyamemazine	363	16.00	4.41
Clozapine	567	13.19	2.33
Flupenthixol	528	21.92	4.15
Haloperidol	197	8.00	4.05
Levomepromazine	243	20.49	8.43
Olanzapine	3563	258.65	7.26
Risperidone	787	6.40	0.81
Tiapride	214	8.42	3.93
Zuclophenthixol	852	168.93	19.82

P- Powder.

RSD - relative standard deviation obtained after four-fold sample processing.



**Table 2**  
Values of recovery assays of the APs tested by titrimetric method.

Antipsychotics	Standard potassium hydrogen phthalate (mg O <sub>2</sub> L <sup>-1</sup> )	Antipsychotic sample (mg O <sub>2</sub> L <sup>-1</sup> )	Antipsychotic fortified (mg O <sub>2</sub> L <sup>-1</sup> )	Recovery rate (%)
Chlorpromazine P	105.6	35.2	131.2	93.2
Chlorpromazine	96	25.6	128	105.3
Cyamemazine	227.2	76.8	300.8	99.0
Clozapine	96	128	211.2	94.3
Flupenthixol	96	105.6	144	71.4
Haloperidol	96	32	131.2	102.5
Levomepromazine	121.6	25.6	70.4	45.8
Olanzapine	121.6	176	217.6	73.1
Risperidone	96	163.2	268.8	103.7
Tiapride	121.6	41.6	144	88.2
Zuclopenthixol	121.6	35.2	92.8	59.2

P- Powder.

levomepromazine and zuclopenthixol. This may be due to the reasons previously exposed on, regarding the fact that these drugs being at very oily solutions it could hinder the homogeneity of the final solutions.

### 3.3. Determination of COD of antipsychotic drugs by sequential injection method

In sequential injection method, a calibration curve was firstly carried out with different concentrations of potassium hydrogen phthalate used as a control, in order to calculate then the percentage change in fluorescence signal.

For each analytical signal, it was given the increase in relation to blank signal (percentage change fluorescence signal). After this, it was used the previously defined potassium hydrogen phthalate calibration curve and determination the value of x which corresponded to mg L<sup>-1</sup> of hydrogen phthalate. Finally, it was converted to mg L<sup>-1</sup> of O<sub>2</sub> using the ratio 1/1.18 (hydrogen phthalate/O<sub>2</sub>).

The COD values obtained by SIA technique are presented in Table 3. Although it was not used standard dichromate solution in SIA methodology, cerium and the UV lamp were responsible for the oxidation of APs. In fact, the photooxidation is a common reaction amongst sulfur-containing pharmaceuticals like the phenothiazine derivatives. In the photodegradation aspect, one of the most studied group of APs were phenothiazine derivatives (Trawinski and Skibinski, 2017). The photolability of phenothiazine derivatives is a well-established fact. For example, the irradiation of chlorpromazine with UV-C and UV-A promoted its significant loss (Prohotsky et al., 2014) and a very extensive photodegradation with use of a xenon lamp was reported by Trautwein

**Table 3**  
APs COD values as well as the respective Standard Deviation and the RSD obtained by the SIA system.

Antipsychotics	Mean (mg O <sub>2</sub> L <sup>-1</sup> )	Standard Deviation (mg O <sub>2</sub> L <sup>-1</sup> )	RSD (%)
Chlorpromazine P	87	12.66	14.50
Chlorpromazine	96	5.03	5.26
Cyamemazine	153	2.65	1.73
Clozapine	118	0.71	0.57
Flupenthixol	124	0.71	0.57
Haloperidol	97	5.66	5.83
Levomepromazine	120	2.08	1.74
Olanzapine	221	4.04	1.83
Risperidone	166	10.66	6.44
Tiapride	112	2.12	1.90
Zuclopenthixol	190	2.08	1.09

P- Powder.

RSD - relative standard deviation obtained after four-fold sample processing.

**Table 4**  
Comparison of the COD values obtained by the both techniques.

Antipsychotics	Mean COD titration method (mg O <sub>2</sub> L <sup>-1</sup> )	Mean COD SIA (mg O <sub>2</sub> L <sup>-1</sup> )	Ratio SIA/ titration method	Ratio titration method/ SIA
Chlorpromazine P	149	87	0.58	1.71
Chlorpromazine	186	96	0.52	1.94
Cyamemazine	363	153	0.42	2.37
Clozapine	567	118	0.21	4.81
Flupenthixol	528	124	0.23	4.26
Haloperidol	197	97	0.49	2.03
Levomepromazine	243	120	0.49	2.02
Olanzapine	3563	221	0.06	16.12
Risperidone	787	166	0.21	4.74
Tiapride	214	112	0.52	1.91
Zuclopenthixol	852	190	0.22	4.48

P- Powder.

and Kummerer (2012). In this sense, low COD values achieved for the phenothiazine derivatives (Table 3) are in agreement with their photolability. On the other hand, most of the studied atypical APs showed to be photostable and it included clozapine, risperidone, and olanzapine (Trawinski and Skibinski, 2017). These findings could be also related to the higher COD values achieved for the atypical or second-generation APs. Therefore, it can be assumed that the results could be influenced by differences in photobleaching rates of different AP drugs.

A potential positive aspect of the photolysis process is the decomposition of psychotropic drugs released to the environment and, as a consequence, decrease of their toxicity. However, this process may lead to the formation of intermediates which can be more toxic than the parent compound. So, it is important to evaluate the photodegradability of these drugs to improve the treatment of wastewater treatment plants (Trawinski and Skibinski, 2017).

### 3.4. Comparison between the COD values by titration method and sequential injection analysis (SIA)

Analyzing the results presented in Table 4, it can be seen that AP drugs have different, but correlated, values of COD in both techniques.

This can be justified through the different oxidation reaction times in both techniques since that in the titrimetric method the contact time between the sample and the oxidizing agent were 2 h and in SIA were only 3 min of photooxidation. This difference in relation to the contact time with the oxidizing agent may interfere with the results of both techniques.

According to what it was described in 3.1, it seems to be possible to cluster APs with the ratio obtained between the methodologies.

So, the APs that have lower COD value, were integrated into the group that had a ratio between titrimetric method/SIA equal to 2. In the case of APs that have higher COD values, most of them had a ratio between titrimetric method/ SIA equal to 4.

Regarding these findings, it was possible to define a trend between the lower COD results obtained by the titration and SIA methods (Fig. 3).

In spite of with the SIA system it was obtained lower COD values than with the titrimetric method, it yields the same type of correlation with the batch procedure. Furthermore, when plotting the APs COD values determined using the SIA method against the COD values determined using the titrimetric method that had a ratio near to 2, a linear relationship was obtained ( $R^2 = 0.98$ ) and the calculated Pearson coefficient ( $\sim 0.99$ ) showed that a good correlation is achieved between the two sets of results obtained by the proposed methodology and by the comparison procedure.

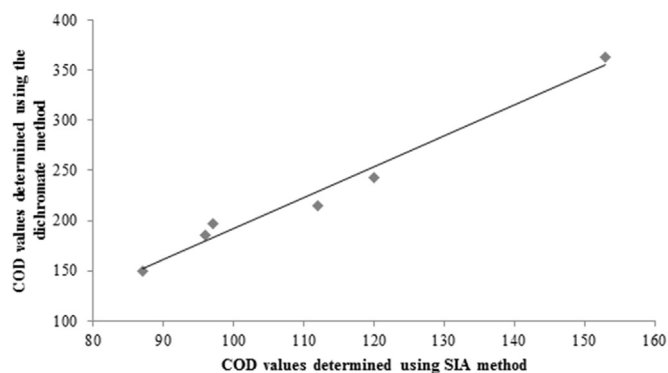


Fig. 3. Comparison of the APs COD values ( $\text{mg L}^{-1} \text{O}_2$ ) with a ratio near to 2 obtained by the both techniques.

#### 4. Conclusions

To the best of our knowledge, this is the first time that it is evaluated the COD values for AP drugs. Considering that the consumption of these psychiatric drugs is rising and as a result, they are continuously being released into the environment, it is imperative to implement methods that monitor their environmental pollution. The obtained COD values for the different APs, both from first and second generation, showed that there is a chemical structure dependency, where the existence of aromatic rings and oxidable groups contribute to higher COD values.

It is also possible to acknowledge from the results obtained that the second generation APs (risperidone, olanzapine, zuclopenthixol, and clozapine) have higher COD values than the first generation APs (chlorpromazine, cyamemazine, levomepromazine). In this way, it will be important to perform more studies in order to evaluate the SAR with the effect of this drugs at the environmental level.

The proposed SIA methodology, based on photooxidation (irradiation with ultraviolet (UV) light), was also successfully applied, with lower COD values but proportionally related to those obtained by the comparison titrimetric procedure.

When evaluating the two methodologies, the SIA methodology is perfectly adequate for high-throughput screening, enabling the results in a short period (3 min in opposite to a 2 h-digestion step followed by a titration) and in a reproducible manner, besides its environment-friendly nature due to the low volumes involved. Compared to other techniques, such as chromatography, it is simpler, not requiring qualified manipulation. With automation there is also a prevention of errors associated to human manipulation and an increase of cost-effectiveness.

On the other hand, when the titrimetric method is compared with another detection method, for example, colorimetric method, the titrimetric method can be used in samples with high turbidity and residual color while in the colorimetric method this is not possible.

It is envisioned that this work opens promising perspectives in implementing complementary methods for the assessment of the effectiveness of the pharmaceutical residues treatment processes, and even its improvement, by exploiting the photoreactivity property of pharmaceuticals.

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