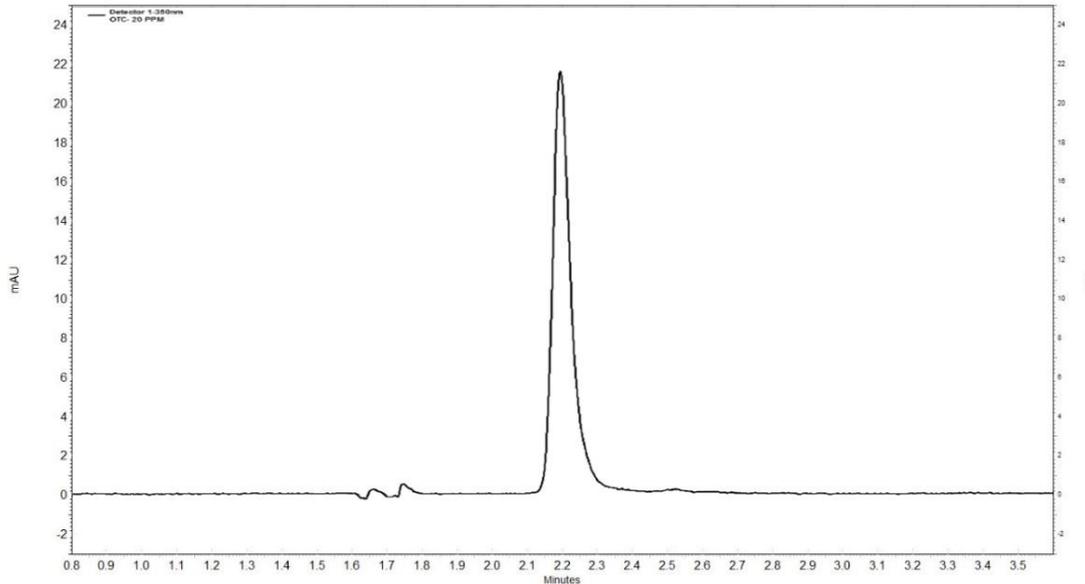


1 **Appendices - Supplementary materials**  
 2 **Appendix A Validation of analytical parameters:**  
 3 *App-A.1 Analysis by HPLC*

4 The OTC present in aqueous solution was detected at a wavelength of 354 nm. presenting a  
 5 retention time of 2.18 minutes. The signal the chromatography obtained can be seen in Figure A1.



6  
 7 **Figure A1.** Chromatogram obtained for the standard of OTC at 354 nm.

8 Based on the results described in Table A1. the average of the areas for the six curves was  
 9 calculated. as well as the standard deviation between them. In addition, it was possible to evaluate  
 10 the dispersion of the results by means of the Grubb's test. This test, note anomalous outliers appear  
 11 larger or smaller than the group measures. Equations A1 and A2 are shown below:

12 
$$G_{<} = \frac{\bar{x} - x_{i<}}{s} \tag{A1}$$

13 
$$G_{>} = \frac{\bar{x} - x_{i>}}{s} \tag{A2}$$

14 Where:  $G_{<}$  = Grubb's test to the lowest measured value;  $G_{>}$  = Grubb's test for the greatest  
 15 measured value;  $\bar{x}$  = Average;  $x_{i<}$  = minor extent;  $x_{i>}$  = superior extent;  $s$  = estimated standard  
 16 deviation.

17  
 18 The results shown in Table A1 indicate that the data are in agreement for the equivalent values  
 19 for the 6 measurements with a 95% confidence level. We can check these results since the values of  
 20 the Grubbs test performed show  $G_{<}$  and  $G_{>}$  lower than 1.822. which is found to be acceptable for the  
 21 data analyzed [1].  
 22  
 23

24 **Table A1** Mean peak area, standard deviation and Grubb's test for OTC in the range of 2 to 20 mg·L<sup>-1</sup>

Concentration (mg·L <sup>-1</sup> )	OTC			
	Areas	SD	G<	G>
2	31248.67	667.55	1.328	0.901
4	59516.00	570.26	1.787	0.905
6	89275.17	4934.25	0.988	1.015
8	120750.67	3246.67	1.189	0.977
10	151649.17	2234.51	0.958	1.198
12	181019.33	5730.73	0.985	0.986
14	206042.17	7653.15	0.940	0.984
16	245533.67	1705.97	1.126	1.583
18	274562.83	7283.15	1.075	0.957
20	307735.00	6016.93	1.161	1.066

25 SD= Standard Deviation; G&lt;= Grubb's lowest; G&gt;=Grubb's largest

26

27 *App-A.II Analysis of linearity*

28 The linearity was determined by calculating the linear regression coefficient of the analytical  
 29 curve (R<sup>2</sup>). Table A2 shows the linear ranges for the OTC. as well as the equation of the line and  
 30 correlation coefficient obtained.

31

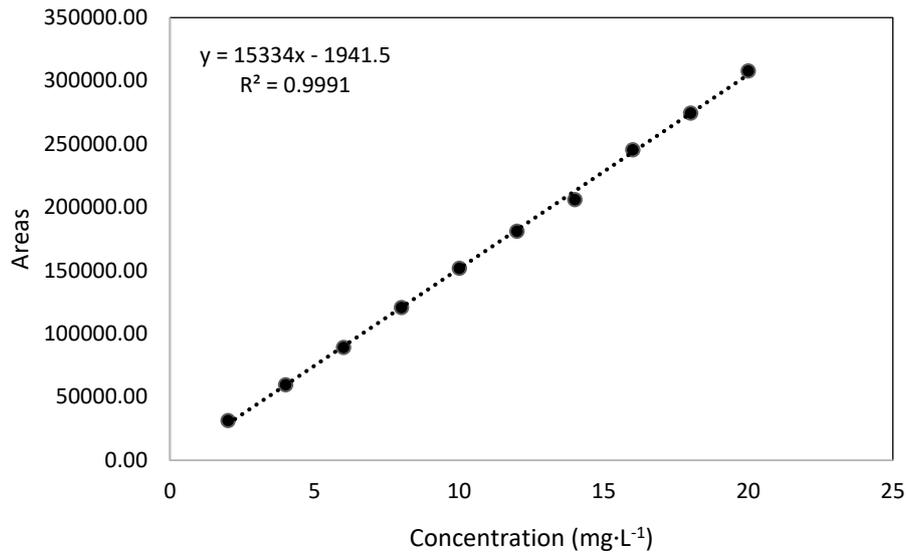
32 **Table A2.** Working range for the compound. straight line equation and determination coefficient (R<sup>2</sup>)

Compound	Linear range (mg·L <sup>-1</sup> )	Equation of the line	R <sup>2</sup>
OTC	2 to 20	y=15334x-1941.5	0.9991

33

34 Table A2 shows that the values of the respective compounds correlations were above 0.99,  
 35 being in accordance with the standards required by the regulatory agencies [2, 3]. Figure A2  
 36 illustrates the linear range of the method. It is verified that the points used in the construction of the  
 37 analytical curve are within the range linear. Therefore, it can be established that the analytical method  
 38 is linear in the interval analyzed.

39



40  
41

**Figure A2.** Graphical representation of linearity for OTC for the range of 2 to 20 mg·L<sup>-1</sup>

42 *App-A.III Analysis of precision*

43 The analysis was performed with precision based on the measurement of coefficient of variation  
44 (CV). Calculations were performed according to equation A3.

$$45 \quad 46 \quad 47 \quad CV (\%) = \frac{s}{\bar{x}} \cdot 100 \quad (A3)$$

48 Where (s) is the standard deviation estimate and  $\bar{x}$  is the mean of the values analyzed in the  
49 curve.

50 The CV values obtained for each concentration used in the construction of the curve are  
51 described in the Tables A3.

52 Table A3. Values obtained for the linear CV in the range 2 to 20 mg·L<sup>-1</sup>

Concentration (mg·L <sup>-1</sup> )	Coefficient of Variance CV (%)
2	2.1363
4	0.9582
6	5.5270
8	2.6887
10	1.4735
12	3.1658
14	3.7144
16	0.6948
18	2.6526
20	1.9552

53  
54  
55  
56

Coefficients of variance of between 0.69% and 5.53% indicate adequate precision, with values below 20%. [3, 4].

57 *App-A.IV Limit of Detection and Quantification*

58 The LD and LQ were obtained by the method based on the relationship between the standard  
59 deviation of the response and the slope of the curve, according to equations A4 and A5.

60

$$61 \quad LQ = 10 \cdot \frac{s}{s} \quad (A41)$$

$$62 \quad LD = 3 \cdot \frac{s}{s} \quad (A5)$$

63 Table A4. presents the results of LD and LQ for the analytical method for the determination of  
64 OTC in aqueous solution, for a working concentration range of 2 to 20 mg·L<sup>-1</sup>.

65 **Table A4** Limits of detection (LD) and quantification (LQ) of the analytical method

OTC (mg·L <sup>-1</sup> )	Limit of detection (mg·L <sup>-1</sup> )	Limit of quantification (mg·L <sup>-1</sup> )
2 - 20	0.144	0.435

66

67 *App-A.V Analysis of accuracy*

68 The experiments were conducted in triplicate. The calculations for the recovery were obtained  
69 from equation A6.

$$70 \quad R (\%) = \left( \frac{C_1 - C_2}{C_3} \right) \cdot 100 \quad (A6)$$

71 Where: C<sub>1</sub>= concentration determined in the sample with addition of the standard; C<sub>2</sub>=  
72 concentration determined in the sample without addition of the standard; and C<sub>3</sub>= concentration of  
73 added standard.

74 Table A5 details the percentages of recovery obtained for OTC, for this analysis values equal to  
75 or greater than 94% were quantified. According to the literature for complex samples, ranges from 50  
76 to 120% are recommended [3, 5], so the results obtained are within the recommended range.

77

78

**Table A5** Relative Analytical Curve Detection Limits for OTC

Concentration (mg·L <sup>-1</sup> )	OTC
2-4 (recovery)	92.51%
2-8 (recovery)	94.74%
2-12 (recovery)	98.65%
2-16 (recovery)	96.87%
2-20 (recovery)	99.45%

79

80 **References**

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- 91



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