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Chromatographic Method to Determine the Sorption of the Antibiotic Oxytetracycline to Tropical Soils

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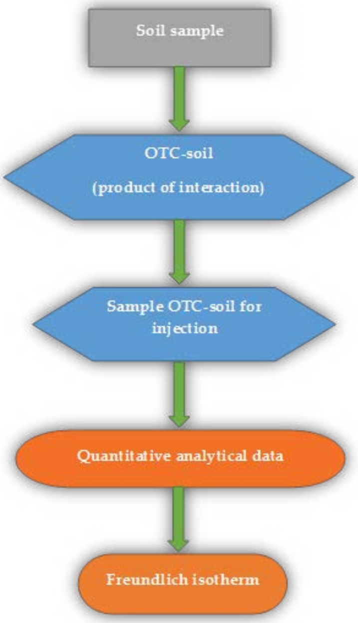
Abstract

This analytical method was adapted to determine the sorption of oxytetracycline (OTC), an antibiotic of large use for animal and agriculture, to tropical soils. Tetracycline antibiotics for veterinary are considered emerging environmental contaminants. The high performance liquid chromatography (HPLC) with UV-visible detector in reverse mode was used to generate quantitative data that allowed the construction of sorption Freundlich isotherms for soils-OTC at pH 4.8. The Freundlich isotherm was presented as a mathematical model suitable for the verification of soils-OTC sorption from chromatographic data, proving the sorption and its type, as well as the soil-OTC sorption capacity. The method consists of:

- Measuring the presence of the oxytetracycline (OTC) veterinary in tropical soils
- Constructing Freundlich isotherm for sorption soil-OTC to understand the OTC's fate on environment



Keywords: Tetracycline antibiotics; Emerging contaminants; Freundlich isotherm; Soil pollutant; Environmental chemistry

Method Details

This analytical method was adapted to determine the sorption of oxytetracycline (OTC), an antibiotic of large use for animal and agriculture, to tropical soils [1]. Tetracycline antibiotics for veterinary are considered emerging environmental contaminants [2]. Actually, analytical methods to determine OTC in soils available in literature were developed for temperate regions, which have different chemical composition and physicochemical properties when compared against soils from tropical regions, as Brazil, where there are large agricultural and livestock activities. The soil-OTC sorption measures was carried out in triplicate using two soils (M1 and T1) from Sao Paulo state from Brazil; M1 (forestry, Oxisol dystrophic - 21°40'4" S and 47°50'33" W) and T1 (peat, Organosol- 21°33'20" S, 47°55'08" W), which were collected in superficial depth (0-15 cm). Soils were previously analyzed for the chemical composition and physicochemical properties before the sorption experiment (supplementary data) - T1. The sorption experiment was adapted from procedure developed by Jones et al. [3]. As the charge and speciation of OTC and soil are pH dependent, opted for a buffering medium (pH 4.8), close to the maximum mass fraction of the OTC Zwitterion and to minimize possible interference of pH and electronic loads for adsorption.

It was previously prepared an OTC solution (Oxytetracycline hydrochloride, HPLC grade ≥ 95% purity, Sigma-Aldrich) in a buffer solution of sodium acetate (Synth)/acetic acid (Synth) 0.1 mol L^{-1} in

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ultrapure water (Milli-Q) from an OTC stock-solution of 240 mg L^{-1}. The following concentrations were prepared: 120, 60, 30, 20, 10 and 5 mg L^{-1}; furthermore, a control solution of 0 mg L^{-1} was prepared. A 10 mL aliquot of each solution was transferred to amber glass flask and then immediately added to the soil mass for an m/v concentration of 5 g L^{-1}. The mixtures were shaken for 48 h, protected from light at room temperature. After this period was collected 5 µL of the supernatant, using a micro-syringe (Hamilton) with filter of PVDF with pore size of 0.22 µm for waste retention, followed by the chromatographic analysis. The results were used for the construction of sorption isotherms (Freundlich isotherm).

For the HPLC analysis, was used an equipment Prostar Varian with a UV-Vis detector and a reverse column of polystyrene-divinylbenzene (Phenomenex) with 15 cm length, 4.6 mm internal diameter and 5 µm particle size, according the operating conditions adapted from Loke et al. [4] to temperate soil:

- Isocratic mobile phase (v/v): 26% of acetonitrile (JT Baker) in 74% of aqueous solution of trifluoacetic acid (Mallinckrodt) at 0.05%, with solutions previously bled in ultrasound bath for 15 min to avoid the presence of air bubbles in the system - acetonitrile had HPLC grade and trifluoroacetic acid had a purity of 99%;
- Column temperature: 30°C;
- Flow of the mobile phase: 1 mL min^{-1};
- Detector wavelength: 355 nm.

An external calibration curve was constructed using seven points: 5, 10, 20, 30, 60, 120 and 240 mg L^{-1}. From this curve it was determined the limit of quantification (LOQ) for the method. LOQ was determined according IUPAC recommendation [5]:

$$LOQ=10 \times s/S \qquad (1)$$

Where *s* is the estimated standard deviation of the response - obtained from the linear regression equation - and S is the slope of the calibration curve. Thus, was determined a LOQ of 4.0 mg L^{-1}, with the method does not presenting significant variations. Figure 1 shows the calibration curve obtained. Freundlich isotherms are constructed to verify the influence of soil type - e.g., the effect of the organic matter content - on the sorption of OTC, choosing soils with different characteristics. The Freundlich isotherm applied to heterogeneous surfaces is defined by:

$$S=k_f Ce^N \qquad (2)$$

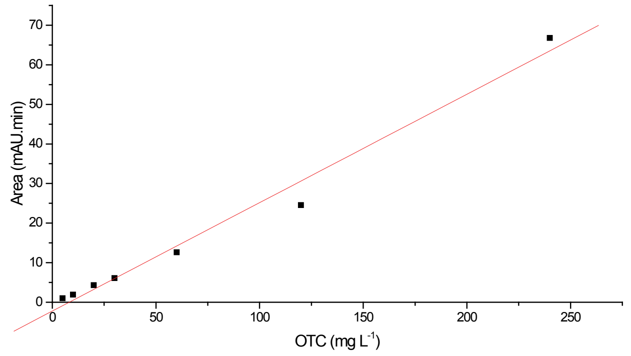

Figure 1: External calibration curve for OTC at pH 4.8 with a correlation coefficient R of 0.99.

Where S is the concentration of solute sorbed in mg g^{-1}, k_f is the sorption coefficient in mg^{1-n} L g^n, C_e is the concentration of solute in equilibrium in aqueous solution in mg L^{-1} and N is the linearity parameter. In a simple case, N=1 and k_f is equivalent to the K_d for linear isotherms. The logarithmic form of the equation is used for the linear fit of the model:

$$LogS=logK_f+NlogC_e \qquad (3)$$

It should be noted that the N value of the Freundlich isotherm allows infer the shape of it and its sorption mechanism, where N=1 represents an isothermal linear C or partition, N<1 one isotherm L and N>1 one isotherm S [6]. The higher value of N, more heterogeneous is the sorption [7]. Based on Giles et al. [8], the physicochemical significances of the isotherms shape are:

- Isotherm C (linear or partition): sorbate (OTC) has a higher affinity for the sorbent (soil) surface than the aqueous phase;
- Isotherm L: sorbate has a high affinity for the sorbent at low concentration; however, in a high sorbate concentration this affinity decreases along with the number of sorption sites;
- Isotherm S: suggests that there is a barrier for the sorbate sorption; if the limitation is overlapped, the sorption is similar to L (above), namely, the sorption increases with increasing concentration of the sorbate in the aqueous phase.

The observed signals obtained by HPLC are due to non-sorbed OTC in aqueous solution or free OTC. It can be defined by:

$$C_f=C_i-C_e \qquad (4)$$

Where C_f is the final OTC concentration or free OTC (non-sorbed) in aqueous solution in mg L^{-1}, C_i is the initial concentration of OTC in mg L^{-1}, and C_e is the concentration of solute in equilibrium in aqueous solution in mg L^{-1} (Equation 2). By means the Equation 4, C_e is calculated for its use in the Equations 2 or 3. Chromatograms in the Figures 2 and 3 show the profile of the sorption soil-OTC for the soil samples M1 and T1. As commented above, the observed signals are due to non-sorbet OTC in aqueous solution.

Table 1 shows the fit parameters from the analyses in the Figures 2 and 3 and from the use of Equations 2, 3 and 4. If the sorption obey the Freundlich isotherm, as in this case, it is by forming multilayers [9] of the sorbed species (OTC) over the surface of the sorbent specie (soil). Then, for both soil samples sorbate had a high affinity for the sorbent at low concentration - isotherm L; however, in a high sorbate concentration this affinity decreases along with the number of sorption sites. Differences in the K_f values are, probably, due to organic matter content, soil granulometry and cationic exchange capacity [3] - according supplementary material.

Summarizing, the steps involved in the method application are:

1. Sample tropical soil in the field - analyze and characterize it for chemical composition and physicochemical properties, respectively;
2. Add OTC for non-real sample or prepare real sample with suspect of OTC presence;
3. Prepare the equipment for the HPLC analysis;
4. Inject standards and samples;
5. Prepare calibration curve taking into account the parameters of validation recommended by IUPAC [5];
6. Use the obtained data to construct the Freundlich isotherm.

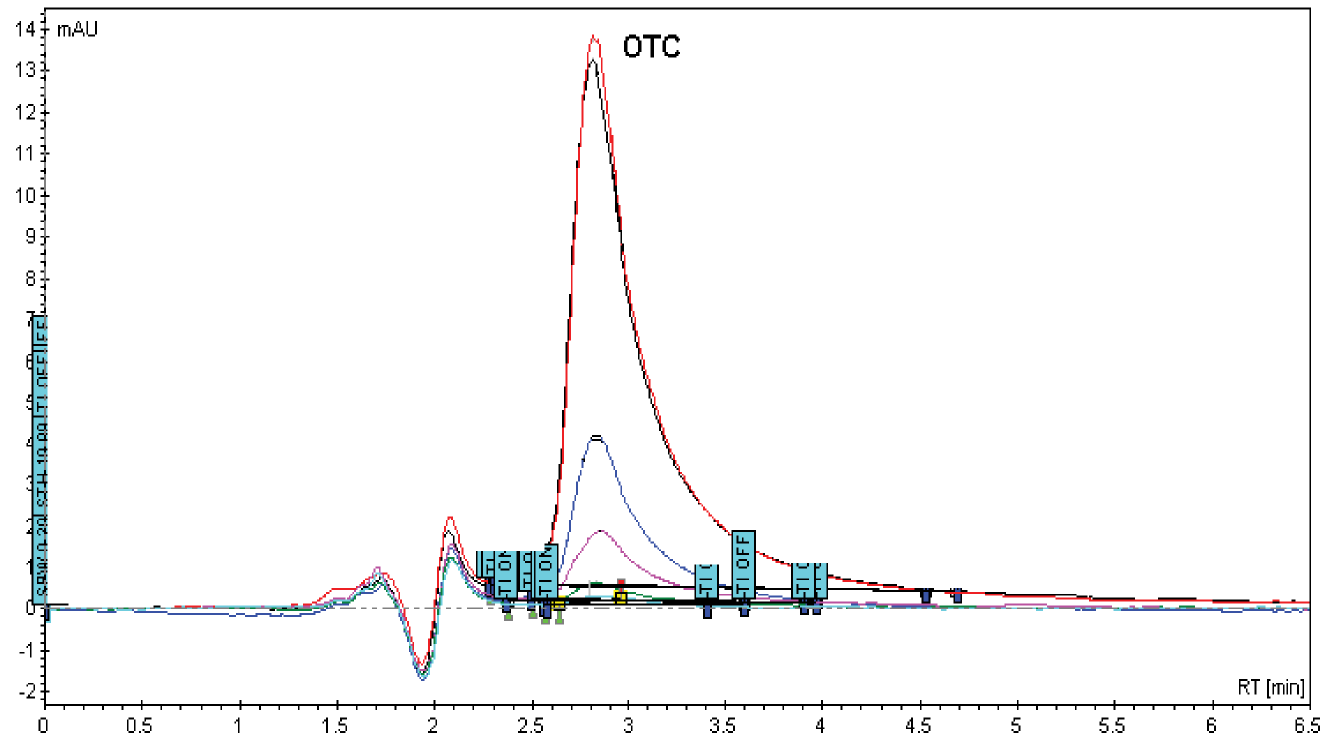

Figure 2: Overlaid chromatograms soil"M1"-OTC obtained at pH 4.8, where: C_i OTC=120 mg L^{-1} (Black line), C_i OTC=60 mg L^{-1} (Red line), C_i OTC=30 mg L^{-1} (Blue line), C_i OTC=20 mg L^{-1} (Pink line).

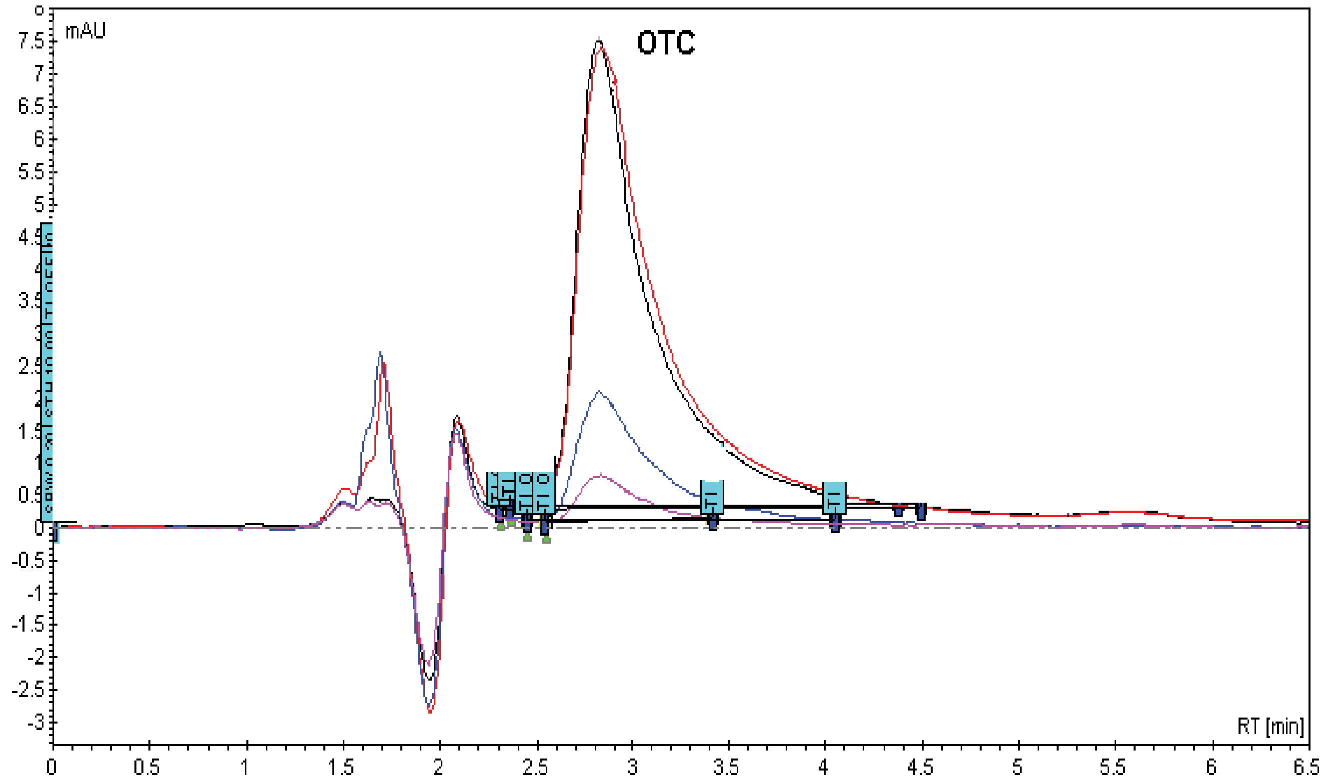

Figure 3: Overlaid chromatograms soil"T1"-OTC obtained at pH 4.8, where: C_i OTC=120 mg L^{-1} (Black line), C_i OTC=60 mg L^{-1} (Red line), C_i OTC=30 mg L^{-1} (Blue line), C_i OTC=20 mg L^{-1} (Pink line).

Freundlich isotherm equation	Value of K_f (mg^{1-n} $L\ g^n$)	Value of N	Isotherm form	R	Soil
$LogS=0.51+0.62logC_e$	3.24	0.62	L	0.928	T1
$LogS=0.39+0.47logC_e$	2.45	0.47	L	0.815	M1

Table 1: Calculated values for the Freundlich isotherms after the linear fit of the equation in its logaritm form. Fit was applied by means the software Origin®. The isotherm form was highlighted as the mean information obtained.

General Comments

The method application should obey the good laboratory practice [10] to ensure the quality and reliability of the obtained data. Furthermore, it is strongly recommended that green chemistry principles [11] are used, as: (1) atomic and energy economy; (2) decreasing solvent use; and (3) a decrease in residues. The application of these principles will contribute to achieve a more sustainable analytical methodology.

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