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Analytical Methods

# Determination of oxytetracycline in tomatoes by HPLC using fluorescence detection

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**Abstract**

An analytical method for the determination of oxytetracycline (OTC) in tomatoes was developed and validated. Liquid–liquid extraction (LLE) and a solid-phase extraction (SPE) were used for sample preparation. Reversed-phase high performance liquid chromatography (RP-HPLC) using a $C_{18}$ column and a mobile phase containing MeOH: calcium chloride, disodium ethylenediaminetetraacetate (EDTA) and sodium acetate, pH 7.3 (30:70, v/v), with fluorescence detection at 390 nm excitation and 512 nm emission, was used for separation and quantitation of OTC. The method was validated through the following performance criteria: linearity and linear range, sensitivity, selectivity, intra-day and inter-day precision, detection and quantitation limits and accuracy. Limit of quantitation show that the method developed is suitable for the determination of OTC at a level below the maximum residue limits established by the Brazilian legislations (250 μg $kg^{-1}$). Of 40 samples analyzed, none contained OTC above the limit of quantitation.


*Keywords:* Tomatoes; Antibiotics; Oxytetracycline; Method validation; High performance liquid chromatography

## 1. Introduction

Antibiotics are the most important bioactive and chemotherapeutic compounds made by microbiological synthesis. They also include antimicrobial compounds present in higher plants and animals. They have proven their significance in varied fields like medicinal chemistry, agriculture and food industry (Kreuzig, 1996). Oxytetracycline (OTC) is a member of the family of the tetracyclines, a group of clinically important natural products and semi-synthetic derivatives characterized by a broad spectrum of activity against pathogenic microorganisms, including gram-positive and gram-negative bacteria and protozoa. These compounds are bacteriostatic antibiotics that act by inhibiting the formation of proteins within the bacterial cell. They are used to control bacterial infections in humans and animals and have also found applications in preserving harvest fruits and vegetables, exterminating insect pests and supplementing animal feed. OTC is produced industrially through fermentation by *Streptomyces rimosus* (Hernández, Borrul, & Callul, 2003; Mitscher, 1978).

Tomato (*Lycopersicon esculentum* Mill.) is an important vegetable crop and is a prone to a number of bacterial diseases, among which bacterial canker disease caused by *Clavibacter michiganensis* ssp. *michiganensis* is one of the most important. Bacterial canker symptoms include vascular wilt, leaf spots and fruit spots. The pathogen attacks other economically important crops including pepper and tobacco (Umesha, 2006).

Some countries, including the United Sates and Brazil, allow the use of OTC in agriculture as fungicide and bactericide to protected crops against pests (Brasil, 1985). Nevertheless, even when applied legally, many antimicrobials leave residues in or on treated food such as fruits, vegetables, grains and other commodities. These residues may remain in both fresh produce (like apples or tomatoes) and processed foods (like applesauce or tomato ketchup).

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In an attempt to address the health issues associated with exposure to these residues, the regulatory agencies set tolerance, or maximum residue limits (MRLs), on the amount of antimicrobial residue that can lawfully remain in or on each treated food commodity.

The Joint FAO/WHO Expert Committee of Food Additives and Contaminants (JECFA), at its 50th Meeting, 1998, established an acceptable daily intake (ADI) of 0–0.03 mg $kg^{-1}$ body weight for the tetracyclines (oxytetracycline, tetracycline and chlortetracycline) alone or in combination. The Committee also recommended maximum residue limits (MRLs) in foods derived from animal species (cattle, pigs, sheep, poultry fish and giant prawn (*Penaeus monodon*)) (WHO, 1998). The MRLs recommended by JECFA have been adopted by the Codex Alimentarius Commission (Codex, 2005). In Brazil, the use of OTC is permitted in the following crop species: tomato, potato, beans, cucumber, coffee, peach, plum, passion fruit and pepper. The maximum residue limit (MRL) established is 0.25 mg $kg^{-1}$ for all commodities, except for plum (MRL 0.7 mg $kg^{-1}$) (Brasil, 1985).

The Environmental Protection Agency of the United States (US/EPA) has established tolerances for residues of oxytetracycline in or on peach and pear (MRL 0.35 mg $kg^{-1}$) (EPA, 2005).

According to Oka, Ito, and Matsumoto (2000), the widespread utilization of tetracyclines leads to an increasing resistance factor, therefore an accurate monitoring by public health agencies and a precise chromatographic analytical method have been required.

Tetracyclines can be successfully determined in various biological matrices, using high performance liquid chromatography (HPLC) in the reverse-phase mode, with different detection modes, such as spectrophotometry, fluorescence and mass spectrometry. The UV detection has low detectability, while mass spectrometry still requires costly instruments. In general, fluorescence detection is sensitive and selective. OTC is known to form chelate complexes with metal ions and the use of chelating agents, such as EDTA and McIlvaine buffer, a mixture of citric acid and $Na_2HPO_4$, to extract OTC from food is commonplace. Microbiological assays are also commonly used for the measurement of tetracyclines in foods, but they are time consuming and their precision appears to be variable (Oka et al., 2000).

The aim of the present study was to develop and validate an analytical method for the determination of OTC in tomatoes by using HPLC with fluorescence detection. The method was applied for the determination of OTC residues in tomatoes purchased from local markets in Alfenas, MG, and Mogi Guaçu, SP, Brazil.

## 2. Experimental procedures

### 2.1. Apparatus

The HPLC system consisted of a Shimadzu LC-10ATvp (Kyoto, Japan) gradient system equipped with a Shimadzu SIL-10AF (Kyoto, Japan) auto injector with a 50 μL loop. The column oven used was a Shimadzu CTO-10ASvp (Kyoto, Japan) operated at 20 °C and the flow rate was 0.8 mL $min^{-1}$. The mobile phase was degassed using a ultrasonic bath (Unique, São Paulo, Brazil). The fluorescence detector was a Shimadzu RF-10Axl (Kyoto, Japan) at 390 nm excitation and 512 nm emission wavelengths. Data acquisition and treatment was performed by a chromatography data system Class-VP (Shimadzu). A BDS Hypersil $C_{18}$ 5 μm column (250 mm × 4.6 mm i.d.) and a similar pre-column (4 mm × 4.6 mm i.d.) from Thermo (Bellefonte, USA) were used for the separation. Vortex mixer used was Certomat MV from B. Braun Biotech International (Melsungen, Germany). Measurements of pH were made with a model NT PH2 pH-meter from Nova Técnica (São Paulo, Brazil), using a combined glass electrode. For solid-phase extraction, octadecyl cartridges $C_{18}$ (500 mg/3 mL) from Unitech (Medley, USA) and CRS (Louisville, USA) and a Visiprep DL vacuum manifold from Supelco (Bellefonte, USA) with 12 ports model were employed.

### 2.2. Standards and reagents

Standards of oxytetracycline hydrochloride and tetracycline hydrochloride were obtained from Sigma (St. Louis, USA). Analytical reagent grade sodium acetate, calcium chloride, disodium EDTA, citric acid, disodium hydrogen phosphate, hydrogen peroxide, sodium hydroxide, hydrochloride acid were supplied from Merck (Darmstadt, Germany). HPLC grade methanol was purchased from J.T. Baker (Philipsburg, USA) and *n*-hexane analytical grade from Merck (Darmstadt, Germany). Throughout the study, water was obtained from a Milli-Q system from Millipore (São Paulo, Brazil). Before analysis, the mobile phase was filtered through a 0.45 μm nylon filter from Millipore (São Paulo, Brazil) under vacuum. The McIlvaine buffer (pH 4.0) and $Na_2EDTA$–McIlvaine buffer (pH 4.0 and 8.0) solutions were weekly prepared as previously described (Pena, Lino, & Silveira, 1999).

### 2.3. Samples

A total of 40 samples of tomatoes belonging to the cultivars of the group Santa Cruz ($n = 15$), Salada ($n = 10$) and Saladinha ($n = 15$) were purchased from local markets in Alfenas, MG, and Mogi Guaçu, SP, Brazil, in March and April 2006. All the samples were taken in accordance with the guidelines of the Commission Directive (2002) and weighed at least 1 kg and consisted of at least ten individual pieces of fruit. All samples were put into plastic bags and transported to the laboratory, were they were immediately subjected to analysis.

Antibiotic-free tomatoes samples used in the method validation were obtained from tomatoes grown in open field in Alfenas, MG, in which the antibiotic was not applied.

### 2.4. Standard solutions

Stock solutions (1 mg $mL^{-1}$) of OTC and tetracycline (TC) were prepared by dissolving 10 mg in 10 mL of methanol. They were kept in brown glass vials in the freezer (−18 °C) and were stable at least 2 months. Working standard solutions in the concentration of 10 μg $mL^{-1}$ were prepared daily by dilution of stock solutions in methanol to appropriate concentrations. These solutions were prepared immediately before use.

### 2.5. Extraction and clean-up procedure

The tomatoes of each sample (1 kg) was chopped, triturated and homogenized in a blender, sifted and a portion of 1 g was weighed and placed into a glass tube and dissolved in 10 mL of 0.10 M $Na_2EDTA$–McIlvaine buffer (pH 8.0). The sample solution was shaken for 3 min in a vortex mixer at high speed. After filtration through a Buchner funnel, the sample was submitted to liquid–liquid extraction (LLE) with 10 mL of hexane in a separatory funnel. The aqueous phase was adjusted to the pH 4.0 with citric acid 0.5 M and was applied to a $C_{18}$ SPE cartridge, 500 mg/3 mL. This cartridge was previously conditioned with 3 mL of methanol and 3 mL of $Na_2EDTA$–McIlvaine buffer (pH 4.0). Then, the cartridge containing the sample was washed with 3 mL of McIlvaine buffer (pH 4.0): methanol (85:15, v/v). The OTC and TC were eluted with 3 mL of methanol. The eluate was concentrated to dryness under nitrogen at 40 °C and the residue was dissolved in 100 μL of the mobile phase. The final eluate was filtered through a 0.45 μm membrane filters Millipore (São Paulo, Brazil) and stirred in vortex before injecting into the chromatograph. An aliquot of 50 μL was injected.

### 2.6. HPLC procedure

The separation conditions for the oxytetracycline and tetracycline were a mobile phase flow 0.8 mL $min^{-1}$ containing an aqueous solution 0.035 M calcium chloride, 0.025 M disodium ethylenediaminetetraacetate (EDTA) and 0.075 M sodium acetate buffered to pH 7.3 and methanol (70:30, v/v) filtered through a 0.45 μm nylon filter under vacuum and degassed by ultrasonication. Column oven temperature was 20 °C and fluorescence detector operated at an excitation wavelength of 390 nm and an emission wavelength of 512 nm. The autosampler was set to inject 50 μL aliquots of samples. The quantitation was accomplished through an internal analytical curve with six concentration levels in the range of 100–350 μg $kg^{-1}$ (OTC) and 250 μg $kg^{-1}$ (TC) as internal standard. Extraction recoveries were determined spiking antibiotic-free fresh tomatoes samples (1 g) with a fortification solution at three different levels: 150, 250 and 350 μg $kg^{-1}$.

### 2.7. Method validation

The method was *in-house* validated using the following performance criteria: linearity and linear range, sensitivity, selectivity, intra-day and inter-day precision, detection and quantitation limits and accuracy. The solutions for calibration and fortification were prepared in methanol and stored at −18 °C. Linearity, linear range, sensitivity and detection and quantitation limits were established through the analytical curves obtained by quintuplicate analysis of OTC at six concentration levels (100, 150, 200, 250, 300 and 350 μg $kg^{-1}$). The internal standard used was TC in the concentration level of 250 μg $kg^{-1}$.

The limits of detection (LOD) and quantitation (LOQ) were obtained from three analytical curves and calculated using the following expression: $LOD = 3\sigma/s$ and $LOQ = 10\sigma/s$ where $\sigma$ is the standard deviation of the response and $s$ is the slope of the analytical curve (ICH, 1996).

The intra-day precision of the method, expressed as the relative standard deviation of peak area measurements ($n = 5$), was evaluated through the results obtained with the method operating over one day under the same conditions, using three different fortification levels: 150, 250 and 350 μg $kg^{-1}$. The inter-day precision was determined at the same concentrations levels, and the analyses were performed for 5 days.

The selectivity of the method was evaluated by exposing OTC and TC at a concentration level of 250 μg $kg^{-1}$, to the following stress conditions: 0.010 and 0.10 M HCl, 0.010 and 0.10 M NaOH, 3% v/v $H_2O_2$ and temperature (55 °C) for 1 h and 0.010 M HCl, 0.010 M NaOH, 3% v/v $H_2O_2$ for 24 h. The solutions were analyzed considering the resolution between analyte and other substances formed during the experiment and the analytical signal before and after the exposure of the analyte to the stress conditions, expressed as recovery (Shabir, 2003).

The accuracy of the method was determined as percent recovery, at three different fortification levels: 150, 250 and 350 μg $kg^{-1}$.

### 2.8. Stability studies

To evaluate the stability of OTC in the matrix, a stability study was performed in a tomato sample grown in open field in which the antibiotic was applied. The plants were subjected to treatment with a commercial product which contains in its commercial formulation the active ingredient oxytetracycline at the concentration of 200 g $kg^{-1}$. The dose employed was 2.0 kg $ha^{-1}$. The amount to be delivered per plant was calculated according the manufacturer and was based on the amount of bactericide that is generally recommended per acre. One day after the application, the tomato sample was carried to the laboratory and it was analyzed over one week period.

## 3. Results and discussion

### 3.1. HPLC conditions optimization

Some authors (Houglum, Larson, & Knutson, 1997; Iwaki, Okumura, & Yamazaki, 1992) recommended the use of a mobile phase containing calcium chloride, EDTA and sodium acetate in order to result a highly sensitive HPLC method for the determination of OTC and TC with fluorimetric detection. $CaCl_2$ is used to produce the fluorescent chelate and EDTA is used to prevent quenching of the fluorescence response by the column.

To optimize the HPLC conditions, the effects of the concentration and the proportion of each additive in the mobile phase, pH and column temperature were investigated. The maximum fluorescence intensity of OTC and TC were obtained with a mobile phase containing an aqueous solution 0.035 M calcium chloride, 0.025 M EDTA and 0.075 M sodium acetate, pH 7.3 and methanol (70:30, v/v). The mobile phases were tested in the pH range of 6.5–7.3 and column temperature in the range 20–40 °C. The isocratic analysis under the conditions described allows the separation of OTC and TC with good resolution at flow rate of 0.8 mL $min^{-1}$.

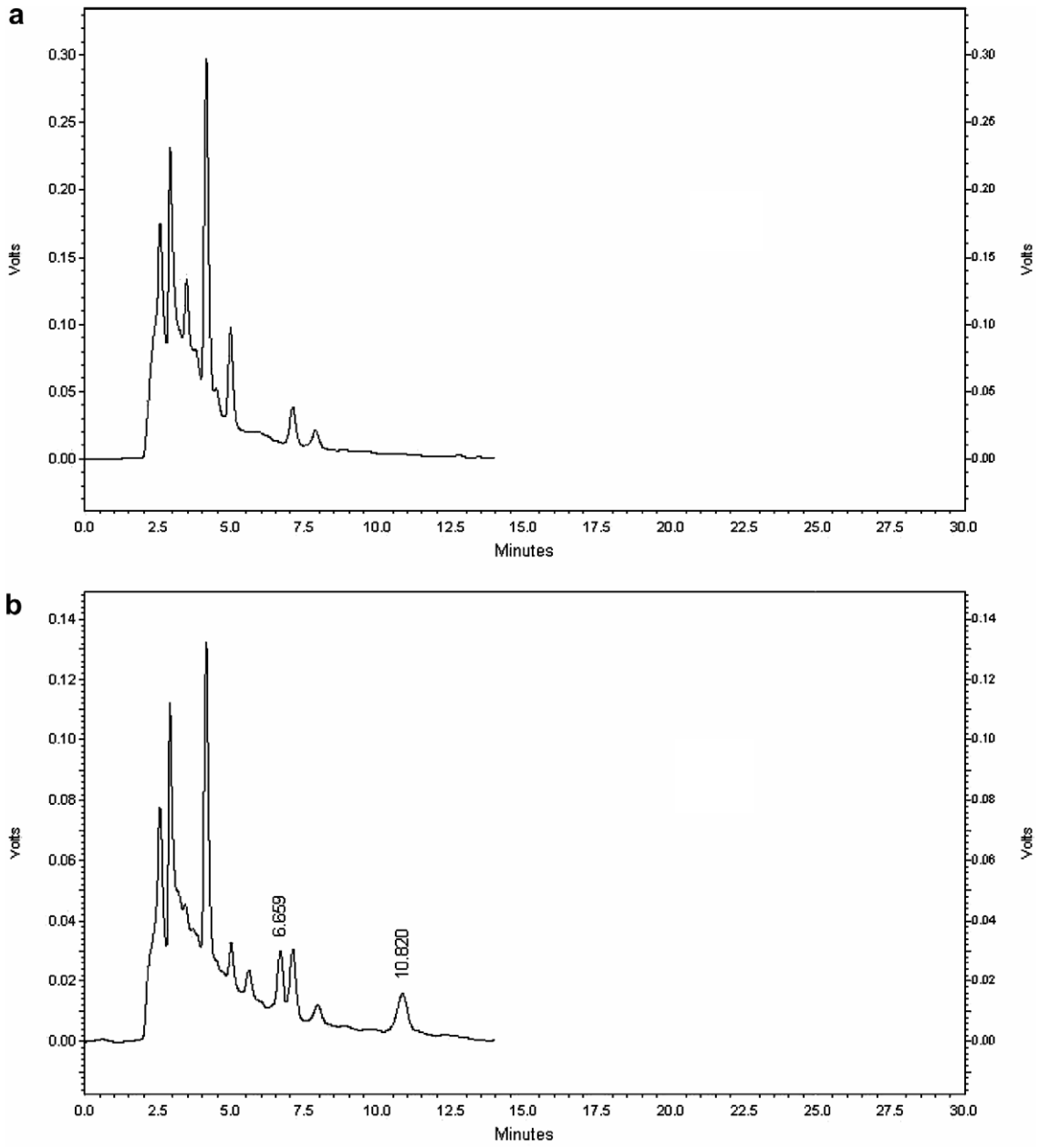


Fig. 1. Chromatograms of the extracts from (a) a known negative tomato sample and (b) spiked tomatoes at fortification level of 250 μg $kg^{-1}$ for OTC and TC using the optimized HPLC conditions. Retention times of OTC and TC: 6.6 and 10.8 min, respectively.

### 3.2. Extraction and clean-up optimization

For solid-phase extraction, initial extractions were carried out using pH 4.0 $Na_2EDTA$–McIlvaine buffer, a mild acidic solvent containing EDTA, which is most commonly used for extraction of tetracyclines from foods. The clean-up efficiencies were studied to adjust the following parameters: the type of clean-up cartridge, the solvents used in the washing steps, the eluent solvent and the volumes for eluting tetracyclines from the cartridge. A number of experiments were then conducted to optimize the extraction recoveries. In order to find the most efficient clean-up method for OTC in tomato, two different commercial octadecyl cartridges $C_{18}$ were compared. In addition, different proportions of the washing solution (McIlvaine buffer pH 4.0: methanol) in the following proportions 80:20, 85:15; 90:10 (v/v) were studied. The hydrogenionic concentration of the $Na_2EDTA$–McIlvaine buffer (pH 4.0 and 5.0) used to conditioning the cartridge was also evaluated. Methanol was chosen to elute the tetracyclines.

Very low recoveries were obtained with these experiments, so a LLE was added to the procedure before the SPE in order to remove some nonpolar compounds of the matrix. Considering the pK values of OTC ($pKa_1 = 3.2$, $pKa_2 = 7.5$ and $pKa_3 = 8.9$) (Anderson, Rupp, & Wu, 2005), in pH 8.0, OTC will be charged and only the nonpolar compounds will be extract by the nonpolar solvent. Thus, 1 g of chopped and sieved tomato was dissolved in 10 mL of 0.10 M $Na_2EDTA$–McIlvaine buffer (pH 8.0). The solution was shaken for 3 min and a LLE with hexane in a separatory funnel for 3 min was conducted. The aqueous phase was taken and the pH adjusted to 4.0. Then, the aqueous extraction phase was applied to a previously conditioned cartridge. The same test were performed with ethyl acetate instead hexane in the LLE procedure. Best results were obtained when hexane was used and the values for recoveries were higher than 70% in fortified samples. One of the two commercial octadecyl cartridges evaluated was chosen because it presented the best results for recovery (>90.0%).

Fig. 1 shows chromatograms of the extracts from (a) blank and (b) spiked tomatoes at fortification level 250 μg $kg^{-1}$ for OTC and TC using the optimized HPLC conditions.

Before analytical method validation, a system suitability test was performed. The following parameters were evaluated: plate count ($N$), resolution ($R_s$), and tailing factor ($T$). The results of resolution and tailing factor obtained were within the acceptable range ($R_s > 2$ and $T \leqslant 2$), according to Shabir (2003). The results are presented in Table 1.

### 3.3. Analytical method validation

The HPLC method was *in-house* validated for the quantitation of OTC in tomatoes by evaluation of the following parameters: linear range, linearity, sensitivity, detection and quantitation limits, intra- and inter-day precision. The results are summarized in Table 2.

Table 1
System suitability parameters for HPLC

| Parameters | OTC | TC |
|---|---|---|
| Plate count ($N$) | 40,867 | 31,424 |
| Resolution[a] ($R_s$) | 8.6 | – |
| Tailing factor ($T$) | 1.0 | 1.0 |
| Retention time (min) | 6.6 | 10.8 |

[a] The resolution was calculated between OTC and TC peaks.

Table 2
Quantitative features for OTC

| Parameter | OTC |
|---|---|
| Linear range (μg $kg^{-1}$) | 100–350 |
| Sensitivity (V μg$^{-1}$ kg) ($P < 0.05$) | 0.0034 |
| Linearity ($r$) | 0.9991 |
| Intercept ($P < 0.05$) | −0.0319 |
| Intra-day precision, $n = 5$ (RSD%) | |
| Fortified at 150 μg $kg^{-1}$ | 5.9 |
| 250 μg $kg^{-1}$ | 5.2 |
| 350 μg $kg^{-1}$ | 6.5 |
| Inter-day precision, $n = 5$ (RSD%) | |
| Fortified at 150 μg $kg^{-1}$ | 8.6 |
| 250 μg $kg^{-1}$ | 10.8 |
| 350 μg $kg^{-1}$ | 11.9 |
| LOD (μg $kg^{-1}$) | 10 |
| LOQ (μg $kg^{-1}$) | 30 |

RSD: relative standard deviation.
$n$ = number of replicates.
LOD: limit of detection and LOQ: limit of quantitation.

The linearity, linear range and sensitivity were obtained from analytical curves using an internal standard (TC) at six concentration levels for the OTC, with quintuplicate analyses. The linearity was tested using a pure error lack of fit test with simple regression, which was not significant at the 5% level.

The precision of the method for OTC was evaluated using the results obtained over 1 day of operation under the same conditions (intra-day) and for 5 days (inter-day). The results are expressed as relative standard deviations (RSD) and are shown in Table 2. Considering that regulatory agencies (Brasil, 2003; GARP, 1999) recommend that the precisions should be up to 15%, the values obtained by the HPLC method are acceptable for both intra- and inter-day evaluations.

The selectivity of the method indicates the ability of the method to accurately measure the analyte response in the presence of all potentially interfering sample components or degradation products (Shabir, 2003). In this study the selectivity was evaluated by exposing the analyte to stress conditions, such as temperature, acid, base and an oxidizing medium. The solutions were analyzed considering the resolution between the analyte and other substances formed during the experiment and the analytical signal before and after exposure of the analyte to the stress con-

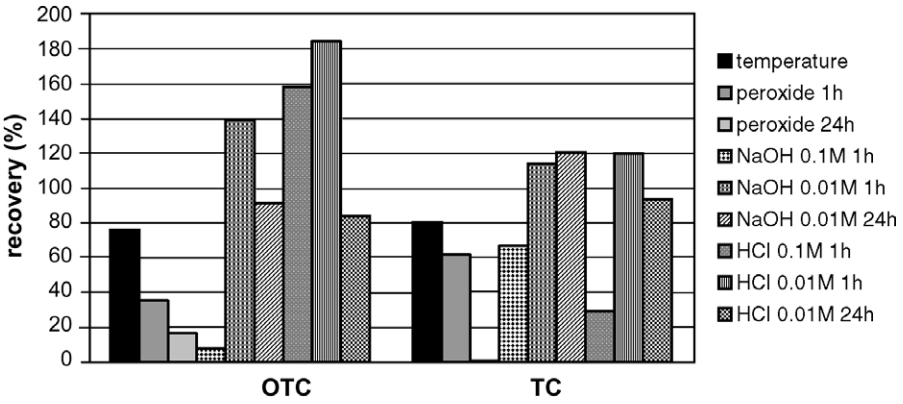


Fig. 2. Recoveries (%) of oxytetracycline (OTC) and tetracycline (TC) after exposure of standard solutions (250 μg $kg^{-1}$) to temperature (55 °C), 0.010 and 0.10 M HCl, 0.010 and 0.10 M NaOH and 3% v/v $H_2O_2$ for one hour and 0.010 M HCl, 0.010 M NaOH and 3% v/v $H_2O_2$ for 24 h.

Table 3
Percentage recovery ($n = 5$) of OTC in fortified tomatoes

| Spiking level (μg $kg^{-1}$) | Recovery mean (%) | RSD (%) |
|---|---|---|
| 150 | 98.0 | 5.8 |
| 250 | 97.9 | 7.8 |
| 350 | 101.9 | 6.6 |

RSD: relative standard deviation.
$n$ = number of replicates.

Table 4
Oxytetracycline levels ($n = 3$) in tomato extracts analyzed over one week period

| Day | Oxytetracycline level (μg $kg^{-1}$) | RSD (%) |
|---|---|---|
| 1 | 292 | 8.4 |
| 2 | 289 | 7.6 |
| 3 | 284 | 7.9 |
| 4 | 153 | 5.5 |
| 5 | 127 | 5.2 |
| 6 | 111 | 6.3 |
| 7 | 95 | 5.1 |

RSD: relative standard deviation.
$n$ = number of replicates.

ditions. The results are presented in Fig. 2. The stability of the analytes depends on their chemical structure and some differences between OTC and TC recoveries were observed. In some conditions (3% v/v $H_2O_2$ for 24 h) of this study, TC degrades almost completely. The degradation products formed under all the stress conditions had retention times significantly different from their corresponding parent analyte, thus confirming the selectivity of the method.

The accuracy of the method was evaluated through recovery test by the analysis of spiked samples with OTC. It was determined as percent recovery and the tomatoes were spiked at different levels: 150, 250 and 350 μg $kg^{-1}$. This parameter was calculated in agreement with ICH (1996). The results are shown in Table 3.

### 3.4. Stability and application to real samples

To evaluate the stability of OTC in the purified extract of tomato an extract containing OTC was stored at 4 °C and analyzed over one week period. The results showed that the analyte was stable on the tomato extract until the third day. The results are summarized in Table 4.

Finally, the HPLC method validated was applied to the determination of OTC in tomato real samples purchased from local markets over different days. A total of 40 samples were analyzed and none of these samples showed contamination of OTC at detectable levels.

## 4. Conclusions

The present paper describes a HPLC method for the determination of oxytetracycline in tomatoes. This method is an accurate and reproducible alternative to the microbial assays that are time consuming. The fluorescence detection is significantly more selective than UV detection and can be used to detect low levels of OTC without additional steps to concentrate the sample.

The results obtained in this work confirm that the HPLC method, when properly optimized and validated, fulfills all the pre-established requirements based on international regulations and show satisfactory recovery values, repeatability and reproducibility and is sensible and specific enough.

The combination of LLE and SPE provides a powerful tool for the determination of OTC residues in fruit and vegetables and the method developed is adequate to detect the antibiotics at concentrations below the LMR established by the Brazilian legislation.

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## References

Anderson, C. R., Rupp, H. S., & Wu, W. (2005). Complexities in tetracycline analysis – Chemistry, matrix extraction, cleanup, and liquid chromatography. *Journal of Chromatography A, 1075*, 23–32.

Brasil (1985). MS/ANVISA Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Portaria No. 10, 08 March 1985 [internet]. <http://e-legis.anvisa.gov.br/leisref/public/showAct.php?id=284&word=> Accessed 08.01.07.

Brasil (2003). MS/ANVISA Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução No. 899, 29 May 2003 [internet]. <http://e-legis.anvisa.gov.br/leisref/public/showAct.php?id=15132&word=> Accessed 08.01.07.

Codex (2005). Codex Alimentarius: Veterinary drug residues in food [internet]. <http://www.codexalimentarius.net/download/standards/45/MRL2_e.pdf> Accessed 08.01.07.

Commission Directive 2002/63/EC of 21 July establishing Community methods of sampling for the official control of pesticides residues in and on products of plant and animal origin [internet]. <http://europa.eu.int/eur-lex/pri/en/oj/dat/2002/l_187/l_18720020716en00300043.pdf> Accessed 08.01.07.

EPA (2005). US Environmental Protection Agency [internet]. <http://www.access.gpo.gov/nara/cfr/waisidx_05/40cfr180_05.html> Accessed 08.01.07.

GARP (1999). Associação Grupo de Analistas de Resíduos de Pesticidas, Manual de Resíduos de Pesticidas em Alimentos (apostila).

Hernández, M., Borrul, F., & Callul, M. (2003). Analysis of antibiotics in biological samples by capillary electrophoresis. *Trends in Analytical Chemistry, 22*, 416–427.

Houglum, J. E., Larson, R. D., & Knutson, A. (1997). Assay of chlortetracycline in animal feeds by liquid chromatography with fluorescence detection. *Journal of the Association of Official Analytical Chemists, 80*, 961–965.

ICH (1996). International Conference on Harmonization. Validation of Analytical Procedures: Methodology Q2B.

Iwaki, K., Okumura, N., & Yamazaki, M. (1992). Determination of tetracycline antibiotics by reversed-phase high-performance liquid chromatography with fluorescence detection. *Journal of Chromatography A, 623*, 153–158.

Kreuzig, F. (1996). Antibiotics in hand book of TLC. In J. Sherma & B. Fried (Eds.). New York: Marcel Dekker (p. 445).

Mitscher, L. A. (1978). *The chemistry of the tetracycline antibiotics. Medical research series* (Vol. 9). New York: Marcel Dekker.

Oka, H., Ito, Y., & Matsumoto, H. (2000). Chromatographic analysis of tetracycline antibiotics in foods. *Journal of Chromatography A, 882*, 109–133.

Pena, A. L. S., Lino, C. M., & Silveira, M. I. N. (1999). Determination of tetracycline antibiotics in salmon muscle by liquid chromatography using postcolumn derivatization with fluorescence detection. *Journal of the Association of Official Analytical Chemists, 82*, 55–60.

Shabir, G. A. (2003). Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the international conference on harmonization. *Journal of Chromatography A, 987*, 57–66.

Umesha, S. (2006). Occurrence of bacterial canker in tomato fields of Karnataka and effect of biological seed treatment on disease incidence. *Crop Protection, 25*, 375–381.

WHO (1998). Toxicological evaluation of certain veterinary drug residues in food. WHO food additives series no. 41 [internet]. <http://www.inchem.org/documents/jecfa/jecmono/v041je07.htm> Accessed 08.01.07.