# Innovative use of microwave radiation in forced degradation of azol pharmaceutical

## Uso inovador de radiação de microondas em degradação forçada de azol farmacêutica

**Fabiana Ernestina Barcellos da Silva[1]*, Cristina Lenhardt Kaefer[1], Natana Becker[1], Fábio de Souza Barbosa[1], Rochele Sogari Picolotto[2], Juliano Smaniotto Barin[3] & Érico Marlon Moraes Flores[2]**

[1] Núcleo de Pesquisa em Fármacos e Medicamentos, Universidade Federal do Pampa – UNIPAMPA – Campus Uruguaiana, BR 472 km 585. Uruguaiana - RS, Brasil.
[2] Departamento de Química, Universidade Federal de Santa Maria. Santa Maria – RS, Brasil.
[3] Departamento de Tecnologia e Ciência dos Alimentos, Universidade Federal de Santa Maria. Santa Maria – RS, Brasil.

**RESUMO**

As micro-ondas são radiações eletromagnéticas usadas em química como fonte de aquecimento. Alguns métodos descritos na literatura usam micro-ondas como fonte de aquecimento em estudos de degradação forçada de produtos farmacêuticos. Neste trabalho, avaliou-se a degradação assistida por micro-ondas (MAD) do levamisol (LEV). A solução de LEV foi aquecida a 100º C em forno micro-ondas por 5-150 minutos. Os resultados demonstraram que cinética por MAD foi de ordem zero e degradação em 150 min foi 73.61 %. Comparando com USP 32 (2009), o método de aquecimento por micro-ondas reduz em 62,2 % o tempo necessário para preparação de amostras para determinação de LEV em matéria-prima ou comprimidos.

**Palavras-chave**: levamisol, estabilidade, produtos de degradação, microo-ondas.

**ABSTRACT**

Microwaves are electromagnetic radiations used in chemistry as heating source. Few methods described in literature use microwave as heating source in forced degradation studies of pharmaceuticals. In this work was evaluated microwave-assisted degradation (MAD) of levamisole (LEV). LEV solution was heated in microwave oven (closed system) in 5-150 min at 100°C. The results demonstrated that MAD kinetics was zero order and degradation in 150 min was 73.61%. Comparing to USP 32 (2009) method microwave heating reduces in 62.2% the time required for sample preparation at LEV determination in raw material or tablets.

**Keywords:** levamisole, stability, degradation products, microwave

*** Corresponding author**: Fabiana E. B. Da Silva. Universidade Federal do Pampa – UNIPAMPA – Campus Uruguaiana, BR 472 km 585. Uruguaiana - RS, Brasil.CEP: 97500220. Tel: +55 55 34134321 Fax: +55 55 34134321 *E-mail address:* fabianasilva@unipampa.edu.br (F. E. B. Silva)

## 1. INTRODUCTION

Microwave radiation has been used in many research labs and routine tests for a wide variety decomposition of samples in some matrices such as soil, hair, milk, diesel oil, biological samples, coal, coke petrochemical, foods, and pharmaceuticals (Bizzi *et al.*, 2011; Silva *et al.*, 2010) . In chemical synthesis, studies involving reactions speed increase by microwave radiation use have shown promising results (Damm *et al.*, 2010; Lill *et al.*, 2007). However, recent publications have been reported in literature using microwave technologies for pharmaceuticals forced degradation/stress studies (Madhavi *et al.*, 2008; Sonawane & Gide, 2011). According to the ICH (International Conference on Harmonization) pharmaceutical forced degradation should be conducted in different media (acidic, basic and oxidizing) at elevated temperatures in order to form degradation products. The objective of this process is: (a) identify the degradation products, (b) propose the molecule degradation route and (c) develop indicating stability methods (ICH, 2003). Furthermore, pharmaceutical forced degradation may be used to prepare solutions resolution for chromatographic routine testing. In this condition the degraded pharmaceutical solution is injected together with the solution containing the intact pharmaceutical and provides resolution between the peaks in a chromatographic system. The main advantage in replacing conventional heating by microwave heating is reducing the sample preparation time. Heating by microwave is a highly efficient process and saves considerable energy, primarily because the fundamental mechanism of microwave involves an agitation of dipolar molecules or ions that could oscillate under the effect of an oscillating electric or magnetic field (Yeh *et al.*, 2007). Some parameters must be considered to propose that replacing, such as degradation kinetics, possible changes in pharmaceutical half-life and degradation products formed (Damm *et al.*, 2010).

The azole pharmaceuticals present in its chemical structure a five membered aromatic ring containing two heteroatoms (where at least one of the heteroatoms must be nitrogen). The core is present in various azole pharmaceuticals and biologically active natural products, since anti-ulcerogenic agents and antifungal agents to molecules which can be used to treat infections. Levamisole hydrochloride (LEV) is an example of pharmaceutical azole containing in its structure an imidazole ring associated to a thiazole ring. It has anthelmintic activity, being particularly effective in infections caused by *Ascaris lumbricoides* (Rang, Dale & Ritter, 2001). According to USP 32 (2009), the method by high performance liquid chromatography (HPLC) proposes conventional heating a basic solution for 5 hours at 100°C to obtain majority degradation product (USP 32, 2009). This solution should be a chromatographic peak with appropriate resolution related to intact pharmaceutical peak. However, this method is slow and uses a large volume of solvent. For routine analysis in pharmaceutical industry, the decrease in analysis time and lower consumption of solvents are required characteristics and which can be achieved by replacing the conventional heating by microwave heating.

This way, this paper describes LEV microwave-assisted degradation (MAD) and provides chromatographic profile products. The aim of this study is to compare pharmaceutical degradation kinetics assisted by microwave and conventional heating.

## 2. EXPERIMENTAL

### 2.1 Chemicals and reagents

LEV was obtained from Henrifarma Ltda. (São Paulo, SP) and tested as described in USP 32 (2009). LEV was certified to contain 100.1% and used without further purification. All the other chemicals and solvents were purchased from commercial sources and used without further purification.

### 2.2 HPLC instrumentation

All analyzes by HPLC were performed on a Shimadzu chromatograph (LC-20AT Prominence), equipped with binary pump, auto injector, diode array detector, column oven and degasser. The analytes were separated on a reversed phase column C18, Macherey-Nagel (MN) (150 mm x 4.0 mm x 5 mm). The mobile phase consisted of monobasic ammonium phosphate buffer 0.05 M (0.3% triethylamine) (Rang, Dale, Ritter, 2001).

### 2.3 Heating equipment

MAD experiments were performed in a *Microwave 3000* microwave reactor equipped with a fiber optic probe for accurate internal temperature measurement (Anton Paar® GmbH, Graz, Austria). Conventional experiments were performed in drying oven.

### 2.4. Procedures

#### 2.3.1 Conventional protocols

Conventional experiments were performed in drying oven at 100 °C (USP 32, 2009). LEV samples (4 mg $mL^{-1}$ in 0.1 M NaOH) were placed in closed flask and heated for 15, 30, 60, 90, 120, 150 and 300 min. Solutions were analyzed by HPLC after dilution in 0.1 M NaOH (160 µg $mL^{-1}$ theoretical concentration). The degradation products were evaluated qualitatively.

#### 2.3.2 Microwave experiments: preliminary degradation

Preliminary degradation was evaluated from time variation (30, 45 and 60 min) and temperature (100 °C and 130 °C) of LEV basic solution. Solutions were analyzed by HPLC after dilution in 0.1 M NaOH (160 µg $mL^{-1}$ theoretical concentration). All experiments were conducted in triplicate. The degradation products were evaluated qualitatively.

#### 2.3.3 Microwave experiments: forced degradation

The MAD was evaluated from variation of time (5, 15, 30, 60, 90, 120 and 150 minutes) at 100 °C of LEV basic solution. In each heating cycle, 10 mL of basic solution were transferred to a quartz tubes, in triplicate. The samples containing LEV basic solution were placed in the same position. In other locations, flasks containing 10 mL of 0.1 M NaOH were used for heat homogeneous distribution. The temperature in each flask was monitored by sensors. In order to confirm degradation products formation, the same procedure described above was carried out at 130 °C. Samples (degraded solutions) were analyzed by HPLC after dilution in 0.1 M NaOH (160 mg $mL^{-1}$ theoretical concentration).

#### 2.3.4 Degradation kinetic assisted by microwave

Degradation kinetics at 100 °C was determined by plotting the graphs (a) concentration x time (zero order kinetics), (b) Logarithm concentration x time (first order kinetics) and (c) inverse concentration x time (second order kinetics). The following kinetic parameters were calculated for degraded samples: reaction order, reaction rate (k), half-life ($t_{½}$) and time to concentration get 90% ($t_{90}$).

## 3. RESULTS AND DISCUSSION

### 3.1 Conventional protocols

Figure 1 shows LEV chromatogram (160 µg $mL^{-1}$) before conventional heating.

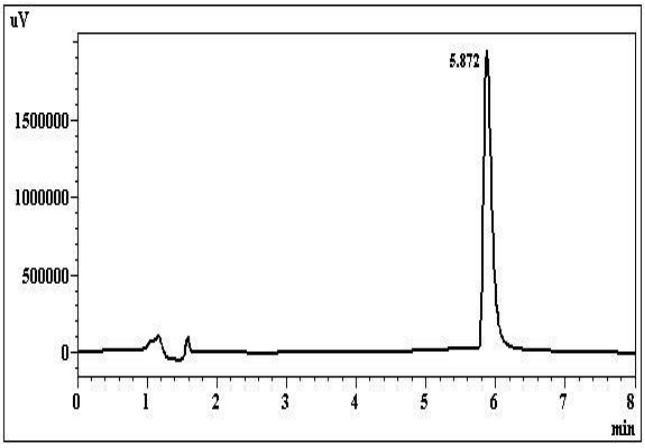


FIGURE 1 – LEV chromatogram

In this condition, the chromatogram of intact pharmaceutical shows only the peak related to LEV at retention time (Tr = 5.9 min). The chromatogram of sample degraded by conventional heating (Figure 2) showed a peak LEV (Tr = 5.9 min) and degradation products in $T_r$ = 6.8 min (PD1) and Tr = 7.8 (PD2). In this condition LEV concentration decrease 58.62% when compared to intact pharmaceutical.

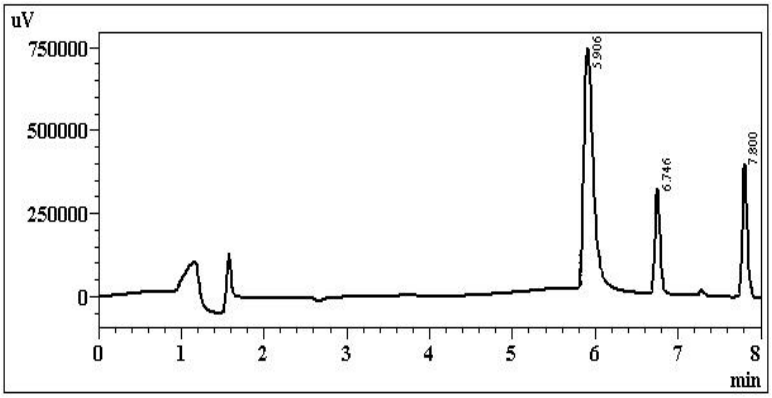


FIGURE 2 – LEV chromatogram after conventional heating (300 min, 100°C)

**3.2 Microwave experiments: preliminary degradation**

LEV was selected as model compound to evaluate the concept of performing microwave-assisted forced degradations in closed systems. As the degradation pathways of LEV are well established and pharmacopoeial monograph is available, analytical thoroughness and a chromatographic method validation was not the main concern in this study (Dickinson, Hudson & Taylor, 1971; USP 32, 2009). Therefore, a precise quantification of degradation products was not carried out rather a comparison of peak areas utilizing HPLC-UV analysis was considered sufficient for the purpose of validating the MAD concept.

According to Brazil (2008), forced degradation test purpose is not completely degrade the compound, but to promote degradation of a small extent (10-30%), sufficient to develop indicating stability methods and identifying degradation products (Brasil, 2008). Thus, a preliminary study of pharmaceutical degradation was conducted to evaluate the temperature at which this condition would be reached. In MAD at 100 °C for 30 min, was obtained 19.3% degradation and no additional peaks were observed after analysis by HPLC. The retention time coincides with that observed previously in LEV degraded by conventional heating. The same profile can be observed for MAD to 100 °C for 60 min, with increased degradation rate in approximately 5%. In this condition there was formation of degradation product in $T_r$ = 6.8 min (PD1). For MAD at 130 °C, degradation reached 80% in 60 min and there was formation of two degradation products (PD1 - $T_r$ = 6.8 min and PD2 - $T_r$ = 7.8 min). In order to observe PD2 formation profile, a study was conducted at 130 °C at different exposure times. In this condition, there was formation PD2 after 15 min and LEV total degradation in 90 minutes (data not shown). The percentage degradation achieved after preliminary degradation can be seen in Figure 3.

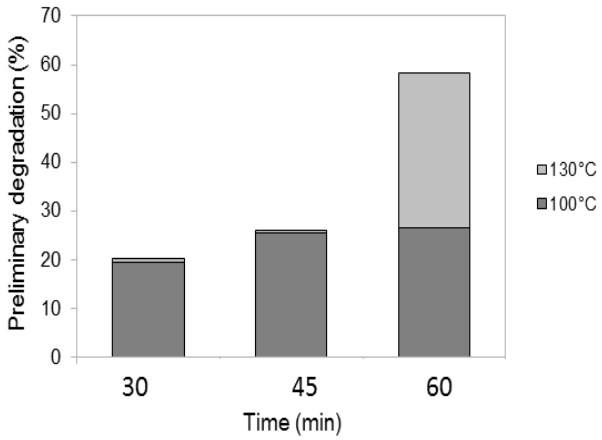


FIGURE 3 - LEV preliminary degradation

The results show that MAD is influenced by time and temperature, directly affecting reaction velocity. Percent degradation equivalent to that obtained by the conventional method (41.38%) was reached for approximately 120 min at 100 °C and 15 min at 130 °C, which shows efficiency in time reduction at microwave heating. As high temperatures typically applied in closed microwave can induce alternative degradation pathways not predictive of degradation pathways at lower

temperatures, 100 °C was chosen for further studies in order to evaluate kinetic degradation (Prekodravak, Damm & Kappe, 2011).

### 3.3 Microwave experiments: forced degradation

In MAD at 100 °C was observed 73.60% degradation at 150 minutes (Figure 4) and formation of degradation product PD1 in 60 min (Figure 5).

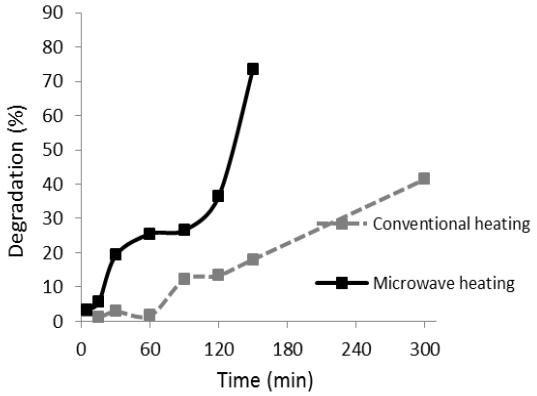


FIGURE 4 – Samples degradation (%) by conventional and microwave heating at 100°C

The peak related to PD1 showed symmetry and excellent resolution in relation to LEV peak. The LEV peak showed no overlap (purity determined by absence of overlapping peaks in chromatographic system with diode array detector). At time 150 min were formed of second degradation product (PD2) at $T_r$ = 7.8 min (Figure 5).

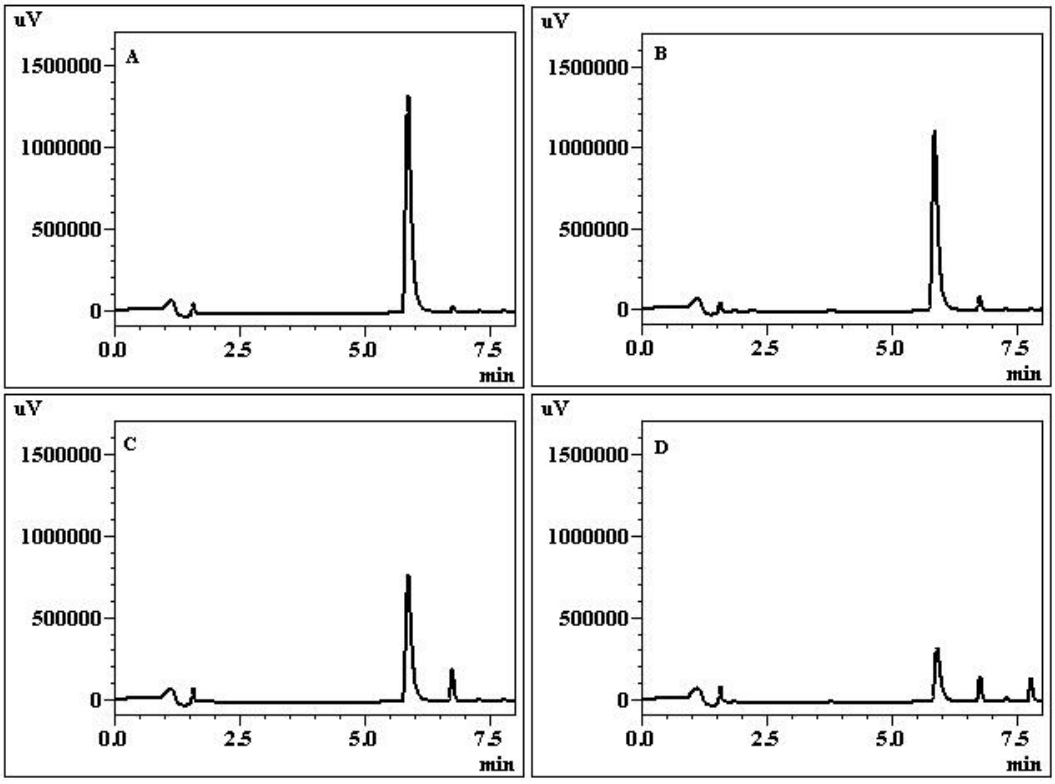


FIGURE 5 – LEV chromatograms after MAD at 100 °C in: (a) 30 min; (b) 60 min; (c) 90 min; (g) 150 min.

This result demonstrates the ability of MAD to accelerate the reaction, without the formation of new degradation products. Compared to conventional heating, microwave heating enhances the rate of certain chemical reactions by 10 to 1,000 times and reactions are cleaner and more environmentally friendly than conventional heating methods. Microwaves heat the compounds directly and usage of solvents can be reduced or eliminated (Gaba & Dhingra, 2011). In sequence, peak areas are showed in Table 1

Table 1 - Peak areas of LEV and degradation products

| Time (min) | Peak area LEV | | Peak area PD1 (6.8 min) | | Peak area PD2 (7.8 min) | |
|---|---|---|---|---|---|---|
| | Coventional Heating | MAD | Coventional Heating | MAD | Coventional Heating | MAD |
| 0 | 13692926 | 13770783 | ----- | ----- | ----- | ----- |
| 150 | 11324945 | 3635693 | 899588 | 692301 | 1913226 | 978199 |
| 300 | 7968458 | NR | 2513468 | NR | 2943260 | NR |

**3.3 Kinetic degradation assisted by microwave**

Studies involving pharmaceutical degradation are useful in order to evaluate concentration decrease in different conditions, in order to establish the shelf life and storage conditions. Thus, by reaction order determination is possible to calculate parameters such as velocity (k), half-life ($t_{1/2}$) time to concentration achieve 90% ($t_{90}$). Furthermore, it is possible by these parameters, comparing kinetics degradation established in different conditions. The values related to concentration versus time at 100° C are shown in Table 2.

TABLE 2 – LEV kinetic degradation

| **Time (min)** | **Concentration (µg mL$^{-1}$)** | |
|---|---|---|
| | **Conventional Heating** | **Microwave Heating** |
| 0 | 160.0 | 160.0 |
| 5 | 155.4 | 154.6 |
| 15 | 158.1 | 151.1 |
| 30 | 155.5 | 129.1 |
| 60 | 157.3 | 119.2 |
| 90 | 140.5 | 117.5 |
| 120 | 138.7 | 101.7 |
| 150 | 131.2 | 42.2 |
| 300 | 66.2 | ------- |
| **Linear Equation** | **y = -0.3058x + 167.29** | **y = -0.6384x + 159.44** |
| **r** | **0.96** | **0.94** |

The reaction follow a zero order kinetics, with k = 0.64 µg mL$^{-1}$ min$^{-1}$ ($t_{1/2}$ =125 min and $t_{90}$ = 25 min). For conventional heating was obtained the same reaction order with k = 0.31 µg mL$^{-1}$ min$^{-1}$($t_{1/2}$ =285.44 min and $t_{90}$ = 76.16 min). No changes in reaction order and formation of new degradation products were observed by MAD in place of conventional heating, showing only increase in degradation velocity. In MAD, 113.43 min were required to achieve the same percentage obtained in conventional heating (300 min). Thus, proposed method reduces in 62.2% the time necessary for sample preparation of LEV tablets determination according to USP 32 (2009).

## 4. CONCLUSION

The method proposed proved to be adequate for analysis of LEV pharmaceutical, with the advantage of heating time reducing, processing of many samples simultaneously and reduced use of solvents. This methodology is presented as an alternative to the procedures using conventional heating in studies involving forced degradation of pharmaceuticals. The innovative use of microwave radiation for this purpose allows the automation of workstations with sampling rate higher than the already available on the market for testing stability.


## 5. ACKNOWLEDGEMENTS

The authors thank to FAPERGS for financial support.


## 6. REFERENCES


Bende G, Kollipara S, Kolachina V, Saha R. Development and Validation of an Stability Indicating RP-LC Method for Determination of Imatinib Mesylate. *Chromatographia* 66:859-866, 2007.

Bizzi CA, Flores EMM, Barin JS, Garcia EE, Nóbrega JA. Understanding the process of microwave-assisted digestion combining diluted nitric acid and oxygen as auxiliary reagent *Microchem. J.* 99(2): 193-196, 2011.

Brasil. Agência Nacional de Vigilância Sanitária. Informe Técnico nº 01, de 15 de julho de 2008 - Esclarecimento sobre o item 2.9 do anexo da Resolução RE nº1 de 29/07/2005, que trata do Guia para Realização de Estudos de Estabilidade. Brasília, DF, 2008.

Damm M, Holzer M, Radspieler G, Marsche G, Kappe CO. Microwave-assisted high-throughput acid hydrolysis in silicon carbide microtiter platforms—A rapid and low volume sample preparation technique for total amino acid analysis in proteins and peptides. *J. Chromatogr. A* 1217:7826-7832, 2010.

Dickinson NA, Hudson HE, Taylor P J. Levamisole: Its Stability in Aqueous Solutions at Elevated Temperatures. Part I. Isolation and Identification of Decomposition Products Formed in Aqueous Solutions of Levamisole Stored under Nitrogen and Oxygen at $100^{O}$ C. *Analyst* 96:235-243, 1971.

Flores EMM, Barin JS, Paniz JNG, Medeiros JÁ, Knapp G. Microwave assisted sample combustion: a technique for sample preparation in trace element determination. *Anal. Chem.* 76(13): 3526-3529, 2004.

Flores EMM, Mesko MF, Moraes DP, Pereira JSF, Mello PA, Barin JS, Knapp G. Determination of halogens in coal after digestion using the microwave-induced combustion technique. *Anal. Chem.* 80(6):1865-1870, 2008.

Franje CA, Chang S, Shyu C. Davis JL, Lee Y, Lee R, Chang C, Chou C. Differential heat stability of amphenicols characterized by structural degradation, mass spectrometry and antimicrobial activity. *J. Pharm. Biomed. Anal.* 53:869-877, 2010.

Gaba M & Dhingra N. Microwave Chemistry: General Features and Applications. *Ind. J. Pharm. Edu. Res.* 45:175-183, 2011.

González-Nuñez LN & Cañizares-Macías MP. Focused microwaves-assisted extraction of theobromine and caffeine from cacao. *Food Chem.* 129(4):1819-1824, 2011.

Gürleyük H, Tyson JF, Uden PC. Determination of extractable arsenic in soils using slurry sampling-on-line microwave extraction-hydride generation-atomic absorption spectrometry *Spectrochim Acta B* 55(7):935-942, 2000.

Hoang TH, Sharma R, Susanto D, Di Maso M, Kwong E. Microwave-assisted extraction of active pharmaceutical ingredient from solid dosage forms. *J. Chromatogr. A*, 1156(1–2):149-153, 2007.

ICH. International Conference on Harmonisation. Stability testing of new drug substances and products Q1A(R2). Current Step 4 version. ICH Steering Committee, 2003.

Lill JR, Ingle ES, Liu PS, Pham V, Sandoval WN. Microwave-assisted Proteomics. *Mass Spectrom. Rev.*, 26:657–671, 2007.

Madhavi A, Reddy GS, Suryanarayana MV, Naidu A. Development and validation of a new analytical method for the determination of related components in tolterodine tartarate using LC *Chromatographia* 68:399–407, 2008.

Mutyala S, Fairbridge C, Paré JRJ, Bélanger JMR, Ng S, Hawkins R. Microwave applications to oil sands and petroleum: A review. *Fuel Proc. Technol.* 91(2):127-135, 2010.

Prekodravak B, Damm M, Kappe CO. Microwave-assisted forced degradation using high-throughput microtiter platforms. *J. Pharm. Biomed. Anal.* 56:867-873, 2011.

Proestos C & Komaitis M. Application of microwave-assisted extraction to the fast extraction of plant phenolic compounds. *LWT - Food Sci. Technol.* 41(4): 652-659, 2008.

Rang HP, Dale MM & Ritter JM. Farmacologia. 4ed. Rio de Janeiro: Guanabara Koogan, 2001.

Silva MJ, Paim APS, Pimentel MF, Cervera ML, Guardia M. Determination of mercury in rice by cold vapor atomic fluorescence spectrometry after microwave-assisted digestion. *Anal. Chim. Acta* 666 (1-2):43-48, 2010.

Sonawane S & Gide P. Optimization of forced degradation using experimental design and development of a stability-indicating liquid chromatographic assay method for rebamipide in bulk and tablet dosage form. *Sci. Pharm.*79: 85–96, 2011.

United States Pharmacopeia: USP 32/NF27. Rockville: U.S. Pharmacopeial Convention, 2009.

Yeh HJ, Lin WC, Sheu SC, Shih TS, Chen LH, Chang HY. Microwave-assisted acid hydrolysis of toluene diamine conjugates in urine samples for biomarkers in toluene diisocyanate analysis. *Talanta* 72:1527-1532, 2007.