

***N,N*-diethyl-*meta*-toluamide (DEET) in repellent solutions: development and validation of an analytical method**

***N,N*-dietil-*meta*-toluamida (DEET) em soluções repelentes: desenvolvimento e validação de um método analítico.**

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**RESUMO**

Um método de análise simples e rápido, apropriado para o controle de qualidade das soluções repelentes que contêm DEET, foi desenvolvido e validado por cromatografia líquida de alta eficiência. Empregou-se a eluição isocrática em coluna Nucleosil 7 C<sub>18</sub>, fase móvel composta de metanol e água (57:43 v / v), fluxo de 1.0 mL/min e o analito foi monitorado a 220 nm. A faixa de linearidade foi 15-75 µg/mL, o desvio padrão relativo (DPR) foi 0.77% para a precisão intra-dia e 1.31% para a precisão inter-dia. As recuperações foram 100.2 e 102.0%. O método mostrou-se robusto quando aplicado a um sistema de cromatografia equivalente e os excipientes não mostraram interferência no tempo de retenção do DEET, dado que conferiu especificidade ao método.

**PALAVRAS CHAVES:** Repelentes de insetos; *N,N*-dietil-3-metilbenzamida; CLAE; Análise quantitativa; Métodos de controle de qualidade.

**ABSTRACT**

A simple and rapid analytical method, appropriate for quality control of repellent solutions containing DEET, was development and validated by high performance liquid chromatography. It was based on an isocratic elution in a Nucleosil 7 C<sub>18</sub> column using a mobile phase composition of methanol and water (57:43 v/v); flow rate of 1.0 mL/min and the analyte was monitored at 220 nm. The linearity ranged between 15-75 µg/mL, the relative standard deviation (RSD) was 0.77% for intra and 1.31 % for inter-day precision. Recoveries of 100.2 and 102.0%. were observed. The method demonstrated to be rugged when applied in an equivalent chromatographic system and the excipients showed no interference in DEET retention time, hence specific.

**KEYWORDS:** Insect repellents; *N,N*-diethyl-3-methylbenzamide; HPLC; Quantitative analysis; Quality control method.

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## INTRODUCTION

DEET (Figure 1) is named N, N-diethyl-meta-toluamide by the IUPAC and N, N-diethyl-3-methylbenzamide by the CAS and it is a controversial repellent substance. It is effective against a broad spectrum of insects, including the yellow fever mosquito (*Aedes aegypti*), the salt-marsh mosquito (*Aedes taeniarhynchus*) and the common malaria mosquito (*Anopheles quadrimaculatus*) (Qiu 1996). Despite being associated with adverse effects in humans, DEET is still the most used repellent worldwide (Osimitz *et al.*, 2010). Studies demonstrated the safe use of DEET-based repellent when applied as recommended (Koren, 2003). Hence, quality control of these products becomes essential.

For quantification of DEET in raw material and pharmaceutical forms, the United States Pharmacopeia includes an infrared technique (United States Pharmacopeia, 2007). Furthermore, of the use of carbon disulphide, the quantitative analysis by infrared has several shortcomings (Skoog *et al.*, 2000). Qui *et al.* (1996) reported one method using solid-phase extraction and LC analysis for quantification of DEET in plasma. Abu-Qare & Abou-Donia (2001) reported one LC using solid-phase extraction with both, UV detection and elution with gradient for quantification of several insecticides and their metabolites. Only one method has been reported for the determination of DEET and DMP in cosmetic products using HPTLC (Markovic *et al.*, 1999). HPTLC is a relatively expensive technique, on contrary, HPLC is a commonly available in most pharmaceuticals laboratories (Kazakevich & LoBruto, 2007).

A simple, rapid and cost-effective analytical method for quality control is preferred. Therefore, development and validation of a method with these characteristics, is presented here, for quantification of DEET in repellent solutions. The validation was performed accordingly to ICH guidelines (2006) and USP (2007) requirements.

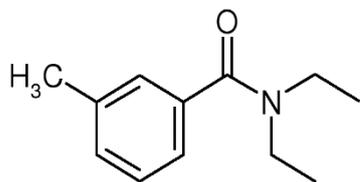


Figure 1. DEET chemical structure

## MATERIALS AND METHODS

### Chemicals

DEET standard substance 99.4% (wet basis), methanol UV/HPLC grade 99.9% from (Sigma-Aldrich, USA) and water purified using Millipore system (18.2 MΩ/cm resistivity, MilliQ™) filtered through Millipore membrane 0.22 μm Millipak™ 40 (Millipore, Corporation™, USA) were used.

### Chromatographic System

The chromatographic analysis was performed in a HPLC-UV Perkin Elmer 200 series equipped with a vacuum degasser. The chromatograms were recorded in a TotalChrom™ version 6.3.1. software. A @ Nucleosil 7 C<sub>18</sub> (250 mm x 4.6 mm id, 5 μm) column, maintained at room temperature (25 °C) was used. The mobile phase composed of 57% methanol and 43% of water; flow rate of 1.0 mL/min; injection volume of 20 μL and detection performed at 220 nm.

### Preparation of solutions

Stock standard solution of DEET (500 μg/mL) was prepared by dissolving 50 mg of DEET in 100 mL of methanol. Dilutions were made to obtain a concentration range of 15-75 μg/mL in mobile phase. The repellent solutions were purchased from the local market and an equivalent of 50 mg of DEET was transferred to 100 mL volumetric flasks and complete with methanol. Aliquots were diluted to final concentration of 50 μg/mL in the mobile phase. The analysis was performed in triplicate and three injections of standard and samples were done.

## RESULTS AND DISCUSSION

### Method development

In the selection of the wavelength, diluted solutions of DEET were prepared in methanol and scanned in the ultraviolet range between 400 nm and 200 nm (Figure 2). C<sub>8</sub> and C<sub>18</sub> columns were tested as well as different proportions of methanol and water for choosing the mobile phase. Chromatographic system performance was assessed by peak symmetry and run time analysis. The asymmetry factor calculated was 0.96 (at 10% of the maximum height of the peak), which demonstrated a symmetrical peak and consequently the appropriated column (Snyder *et al.*, 1997 a). The run time analysis of approximately 7 minutes was suitable; it is recommended between 5 and 10 minutes (Snyder *et al.*, 1997 b).

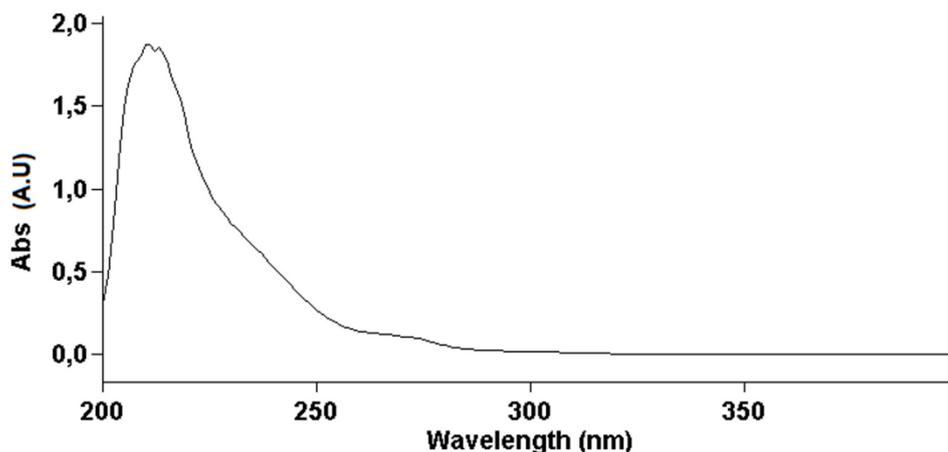


Figure 2. Scanning UV-visible spectrophotometric

### Method validation

#### Linearity

According to International Conference of Harmonization (2006) five different concentrations were prepared, for this study in the range of 15-75 µg/mL in mobile phase. Each concentration was evaluated three times. The linear regression analysis showed a correlation coefficient of 0.9997 resulting in the equation  $y = (52129 \pm 1373) x - (49710 \pm 6481)$ . Through analysis of variance can be concluded that the regression equation is highly significant ( $p < 0.01$ ) and approximately 99.94% of the variation of the peak area can be explained by a linear relation with the concentration of DEET ( $r^2 = 0.9994$ ).

#### Detection and quantification limits or sensitivity

The limit of detection (LD) and quantification (LQ) was calculated based on the standard deviation of the response and the slope, both parameters obtained from analytical curves. The results were 0.0182 µg/mL for LD and 0.0605 µg/mL for LQ.

#### Precision and accuracy

In this study the intra-day (repeatability) and inter-day precision (intermediate precision) were evaluated. For repeatability six determinations in real samples were performed, during the same day and under the same experimental conditions. Intermediate precision was evaluated by analyzing samples in triplicate on different days. Table 1 shown the results expressed as percentage relative standard deviation (% RSD) and the values were within the acceptance criteria.

Table 1. Results of intra-and inter-day precision

	Precision	Average	%RSD
Intra-day n=6		52.45	1.31
Inter-day n=3		52.40	0.77

In order to determinate accuracy of the method, recovery experiments were conducted at three different concentrations. Sample repellent solutions were prepared in methanol to a DEET final concentration of 30 µg/mL and were added to DEET standard solutions to achieve concentrations equivalent to 80%, 100% and 120% of theoretical concentration of DEET. The results were within the specified limits (98% -102%) and showed in Table 2.

Table 2. Results of recovery experiments

Concentration level	DEET theoretical concentration (µg/mL)	DEET experimental concentration (µg/mL)	%RSD	% Recovery <sup>a</sup>
80%	42.58	42.56	1.85	100.05 ± 0.79
100%	49.67	50.67	3.28	102.00 ± 1.66
120%	57.09	57.85	3.81	101.33 ± 2.21

<sup>a</sup> Average of 6 determinations

### Specificity

This parameter was determined demonstrating no interferences between standard and excipients. The DEET peak it maintains unaffected by the excipients. In Figure 3 is observed the absence of absorbance at the analyte retention time. The proposed method proved to be selective and specific.

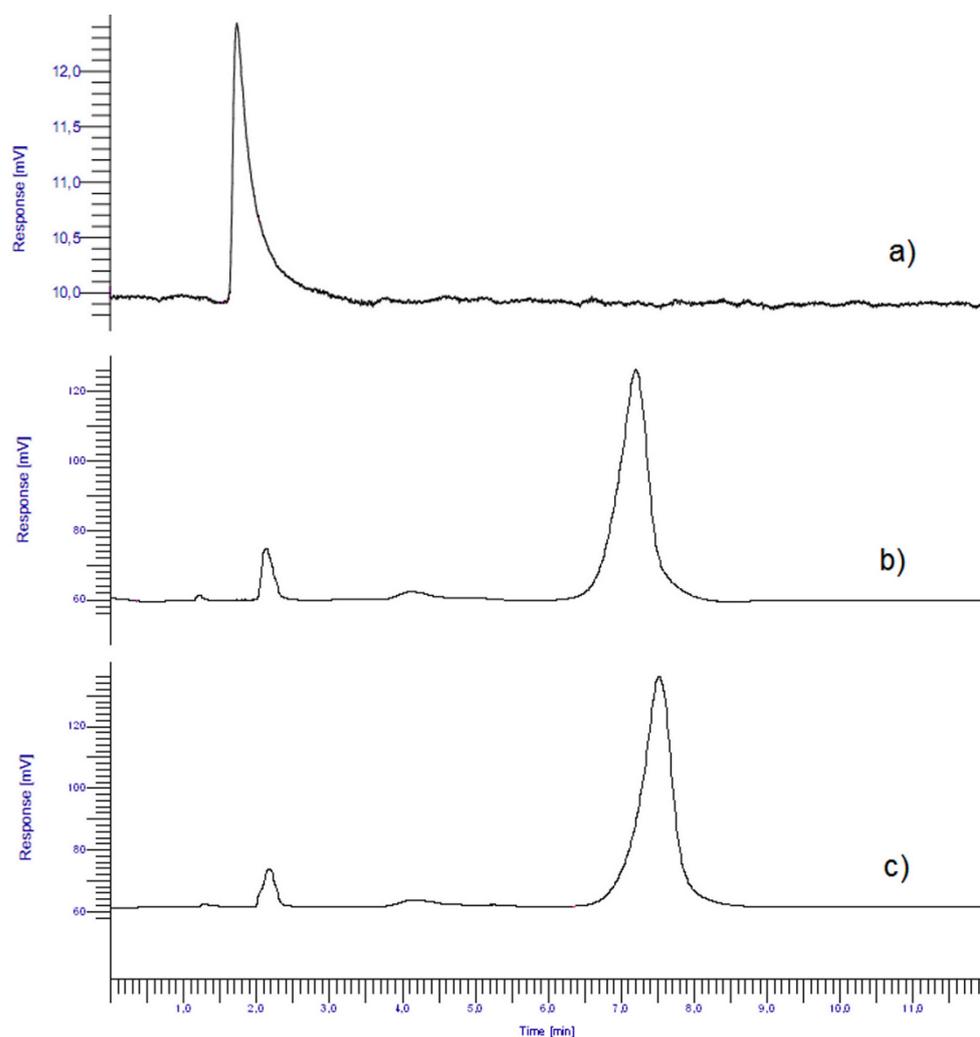


Figure 3. Chromatograms obtained under experimental conditions: a) excipients, b) sample and c) DEET standard solution of 50  $\mu\text{g/mL}$ .

### Ruggedness/Robustness

According to USP cited by LoBrutto & Patel (2007) the ruggedness of an analytical method is the capability of reproducing the results obtained in the same sample by different laboratories, different analysts, different instruments and in different days. Therefore, the method was transferred to an equivalent chromatographic system. The results in Table 3 showed an increase in both, retention time from 7.37 min to 10.07 min and in the asymmetry factor, however the peak symmetry remained within the acceptable values and the quantification was unaffected. The statistical analysis showed no significant differences between the two chromatographic systems, hence the method is considered robust.

Table 3. Results obtained for robustness evaluation

Parameter	Chromatographic System 1	Chromatographic System 2
Retention time (min) <sup>a</sup>	7.37 $\pm$ 0.23	10.07 $\pm$ 0.03
Assay at 100% <sup>a</sup> ( $\mu\text{g/mL}$ )	52.40	52.26
%RSD	0.77	0.86
Asymmetry factor <sup>b</sup>	0.96	1.47

<sup>a</sup> Average of 3 determinations

<sup>b</sup> Obtained at 10% maxim peak height.

## CONCLUSION

The proposed method is rapid, simple and uses equipment and reagents normally available in most quality control laboratories. The technique showed to be linear, accurate, precise, specific and robust; hence it can potentially be used reliably by other laboratories. The method represents a good alternative for quantification of DEET in repellent solutions.

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