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Comparison of HPLC-DAD and LC–MS/MS for the determination and validation of pyriproxyfen in water solutions

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Abstract

Pyriproxyfen is an insect growth regulator that affects the physiology of morphogenesis, reproduction and embryogenesis of insects. The molecule of pyriproxyfen bears little resemblance to endogenous insect juvenile hormone (JH), but it affects JH and ecdysteroid titers in a variety of arthropods. High-performance liquid chromatography with diode array (HPLC-DAD) method is a widely used method for the analyses of pyriproxyfen. Although the methods have been remarkably improved, tandem mass spectrometry (LC–MS/MS) systems with significant advantages have gradually replaced HPLC-DAD in many analyses. The aim of this study, was the evaluation of the two methods for linearity, limit of detection (LOD), limit of quantification (LOQ) selectivity and repeatability for the determination of pyriproxyfen in water solutions. Using HPLC-DAD the obtained LOD was 0.01 µg/ml with the LOQ of 0.03 µg/ml. The linearity was over 0.99 for the concentrations from 0.1 to 1.0 µg/ml with the repeatability RSD less than 11.7%. The LC-MS/MS method showed high reproducibility, as evident from the RSD values for intra-day and inter-day variability being 1.0–6.8% and 2.0–7.7%. The LC-MS/MS method exhibits linearity (R2>0.99) for the concentrations from 1.0 to 100.0 ng/ml with the repeatability RSD less than 12.7%. The obtained LOD and LOQ was 0.1 ng/ml and 1.0 ng/ml, respectively. The HPLC-DAD performed well in terms of various validation parameters, but showed a very high LOD and LOQ (considering low concentration level of pyriproxyfen used in mosquito treatment) compared to LC–MS/MS.

Keywords - HPLC-DAD, LC-MS/MS, pyriproxyfen, water solution

1. Introduction

Pyriproxyfen is the ISO common name for 4-phenoxyphenyl (RS)-2-(2-pyridyloxy)propyl ether (IUPAC), wit the structure shown in Figure 1. Pyriproxyfen is an insecticide that belongs to the class of juvenile hormone mimics with the mode of action - suppression of embryogenesis and inhibition of metamorphosis and reproduction in insects [3]. Owing to its mode of action and safety to nontarget organisms, it is classified as unlikely hazardous category by the World Health Organization [14]. Researchers found that pyriproxyfen is a few hundred times more toxic for mosquito larvae, with LC_{50}=0.011 µg/L and LC_{90}=0.376 µg/L [12] than the most commonly used larvicide temephos. Applied concentration of pyriproxyfen, representative of IGR's, which is sufficient to inhibit the emergence of adult insect genus *Aedes*, in the research is less than 1 ppb [2, 7, 8, 11]. In addition, WHO is committed to its implementation, because the concentration that is sufficient for the inhibition of development of mosquito larvae does not affect the quality of drinking water even if it is found in it [13].

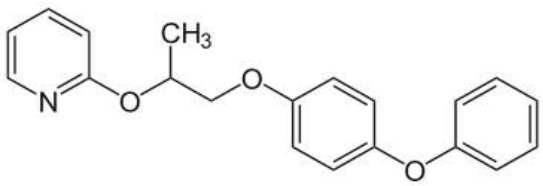

Figure 1. Structural formula of pyriproxyfen

High-performance liquid chromatography with diode array (HPLC-DAD) method is a widely used

method for the analyses of pyriproxyfen [4-6]. Although the methods have been remarkably improved, tandem mass spectrometry (LC–MS/MS) systems with significant advantages have gradually replaced HPLC-DAD in many analyses, also in the determination of pyriproxyfen [15]. In the literature data the use of ultra performance liquid chromatography - tandem mass spectrometry (UPLC-MS/MS) in the analysis of this insecticide can be found [9].

The aim of this study, was the evaluation of the two methods for linearity, limit of detection (LOD), limit of quantification (LOQ) selectivity and repeatability for the determination of pyriproxyfen in water solutions.

2. Material and methods

A. Instrumentation. Agilent 1100 Series HPLC system with Zorbax SB-C18 (5µm, 3.0 x 250 mm) column and DAD. For LC analysis, an Agilent 1200 (Agilent Technologies, USA) HPLC system with a binary pump was used. This was equipped with a reversed-phase C8 analytical column of 150×4.6mm and 5 µm particle size (Agilent Zorbax Eclipse XDB). For the mass spectrometric analysis, an Agilent 6410 Triple-Quad LC/MS system was used. Agilent MassHunter Data Acquisition, Qualitative Analysis and Quantitative Analysis software were applied for method development and data acquisition.

B. Chemicals. The analytical standard of pyriproxyfen of 99.50% purity (Bayer), water of HPLC purity, acetone (GLC-pesticide residue grade, Fisher Chemical, UK), methanol and acetonitrile (HPLC gradient grade, J.T.Backer), HCOOH p.a. (Carlo Erba) were used.

C. Standard solutions. The standard solutions were prepared by dissolving 10 mg ±0.1 of the basic standard in 10 ml of acetone while the working standards were obtained by diluting the basic standard in water. The prepared basic standards were kept in a freezer at -12°C whereas the working solutions were kept in a freezer at 5 °C [10].

D. Instrumentation and chromatographic conditions for HPLC–DAD

The HPLC-DAD conditions were: the mobile phase consisted of water:acetonitrile (70:30 v/v) at 1.0 mL/min, the injection volume 30 µL and the column temperature at 40^{o}C. The detection was carried out at 254 nm.

E. Instrumentation and chromatographic conditions for LC–MS/MS

LC separation was performed on an Agilent 1100 series HPLC system (Agilent Technologies, USA). The system was equipped with a binary solvent pump, an autosampler, and a MS detector coupled with an analytical work station. The MS detector consisted of a multi source that can be configured as APCI (atmospheric pressure chemical ionization) or ESI (electospray ionization). The chromatographyc separation was carried out on a Zorbax Eclipse XDB-C18 column (4.6×100 mm; 1.8 µm) protected by a security guard cartridge C18 (4.6x12,5 mm; 5 µm), both from Agilent Technologies, USA. The separation was performed using gradient elution with methanol as mobile phase A, and water as mobile phase B, both containing 0.1% formic acid. Gradient started with 20% B and it was linearly decreased to 5% B in 10 min and held constantly for 2 min. The flow rate was maintained at 0.5 mL/min.

MS analysis was performed in positive ion modes.The ESI source values were as follows: capillary voltage, 3.5 kV; source gas temperature, 325 °C; vaporizer temperature, 220 °C; desolvation gas (nitrogen, 99.99% purity) flow, 5 L/min. The ideal fragmentation conditions were accomplished varying fragmentation and collision energies for pyriproxifen.

F. Validation parameters. The method was validated in according to SANCO/12495/2011 [10].

The detection limit (LOD) was determined as the lowest concentration giving a response of three times the average baseline. The limit of quantification (LOQ) was determined as the lowest amount of a given pesticide giving a response of ten times the average baseline. LOQ was set based on the regulations and confirmed experimentally by spiking the water sample with pyriproxyfen standard so as to obtain the final mass concentration of 0.03 µg/mL for HPLC. The ratio signal/sound in the obtained chromatograms for the LOQ was calculated on the computer and the LOD values were calculated mathematically. The linearity was checked using matrix matched standards (MMS) at concentrations of 0.1, 0.25, 0.5., 0.75 and 1.0 µg/mL for HPLC analysis and 1.0, 2.0, 5.0, 10 and 20 ng/mL for LC-MS/MS.

3. Results

The comparison of chromatographic methods for the determination particularly low mass concentrations of pyriproxyfen in water solutions, was ceried out on the basis of the optained basic validation parameters.

A. HPLC-DAD. The linearity was over 0.9984 for the concentrations from 0.1 to 1.0 µg/mL with the

repeatability RSD less than 11.7%. The obtained LOD was 0.01 μg/mL with the LOQ of 0.03 μg/mL. The retencion time of this insecticide is 1.37 min (Figure 2).

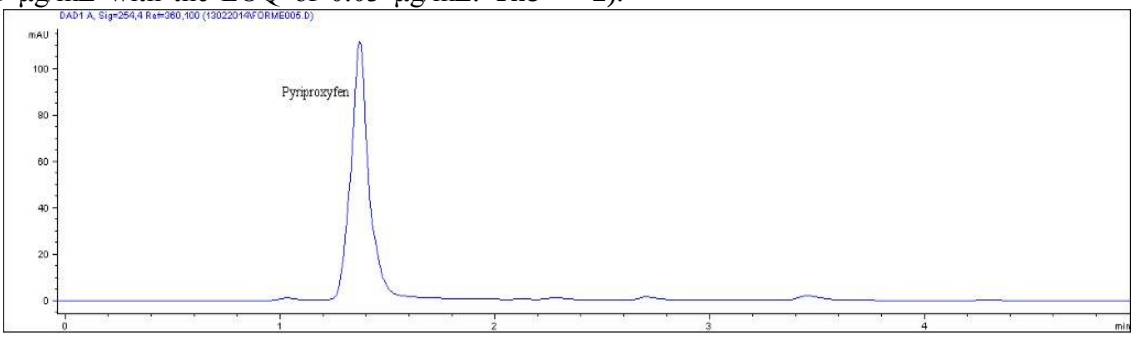

Figure 2. Chromatogram of pyriproxyfen.

B. LC-MS/MS. Table 1 lists the precursor, product ions and the ratio of abundances among both ion transitions as well as the optimized fragmentation energies and collision energies used for MRM. For the detection of pyriproxyfen the precursor ion was m/z 322.1, the product ions selected m/z werw 185.1 and 96.2 (Table 1). Based on the confirmation of parent ions, at least two product ions should be selected in accordance with relevant EU recommendation 2002/657/EC [1] which corresponds to four identification points (one precursor ion and two product ions).

Table 1. MRM, fragmentation and colision energies

Pesticide	***MW (g/mol)***		***Precursor ion* m/z**		***Product ion* m/z**	***Frag* (V)**	***CE* (V)**	***q/Q* (%)**	***Rt* (min)**
Pyriproxyfen	321.369	Q	322.1	→	185.1	100	21	25.5	9.11
		q	322.1	→	96.2	100	13		

Figure 3 shows a LC–MS/MS chromatogram of pyriproxyfen standard solution concentracion 10 ng/mL. The LC-MS/MS method showed high reproducibility as it is evident from the RSD values for intra-day and inter-day variability being 1.0–6.8% and 2.0–7.7%. The LC-MS/MS method exhibits linearity (R^2 = 0.9983) for the concentrations from 1.0 to 20.0 ng/mL (Figure 4) with the repeatability RSD less than 12.7%. The obtained LOD and LOQ were 0.1 ng/mL and 1.0 ng/mL, respectively.

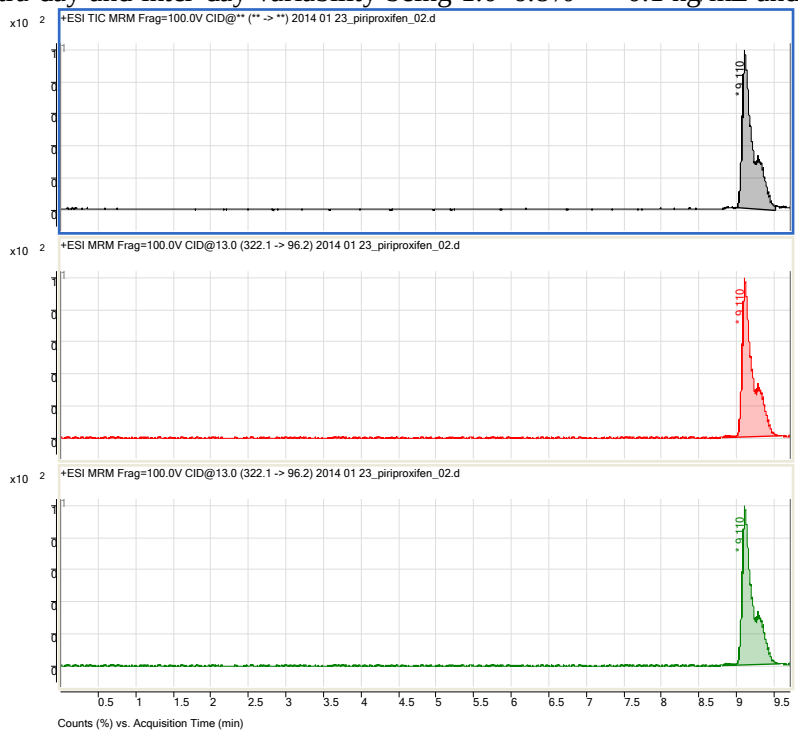

Figure 3. LC–MS/MS chromatogram of pyriproxyfen

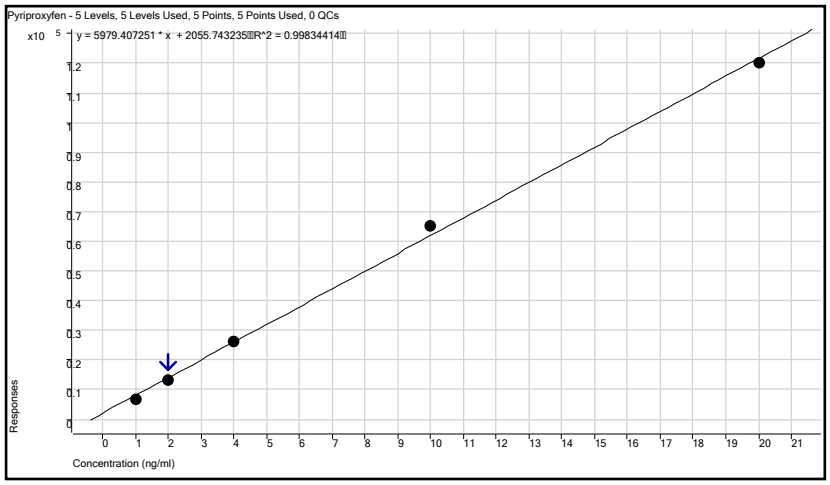

Figure 4. Calibration curve of pyriproxyfen (1.0 -20.0 ng/mL).

4. Discussions

Based on the validation parameters LC-MS/MS method with extremely low LOD and LOQ is more reliable compared with HPLC-DAD (<0.001 μg/mL) considering the fact that the applied concentrations of pyriproxyfena could not be detected by this method. The linearity is the same for the both methods (R^2 = 0.9984 for the HPLC-DAD and 0.9983 the LC-MS/MS). On the other hand, the efficacy of the method which does not decrease by the increase of the flow was taken into account. The principle advantage LC-MS/MS compared with HPLC are a better resolution, nerrow peaks, a higher relation of a signl to noice and the impoved confirmation of the target pesticide. The only advantage of HPLC-DAD is the speed of the analysis it self in wich the retention time of pyriproxyfen is 1.37 min and with LC-MS/MS is 9.11 min which brings obout the decrese of run time.

5. Conclusions

Considering very low concentration level of pyriproxyfen used in mosquito treatment (0.0001μg/ml), a special treatment of mosquito larvae in water, the use of HPLC-DAD is not an adequate technique. On the other hand the use of LC-MS/MS is justified in such research work. The HPLC-DAD performed well in terms of various validation parameters, but showed a very high LOD and LOQ compared to LC–MS/MS.

6. Acknowledgments

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7. References

1. Commission Decision: **2002/657/EC** of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.

2. Estrada J. G, Mulla M. S.: **Evaluation of two new insect growth regulators against mosquitoes in the laboratory**. *Journal of the American Mosquito Control Association* 1986, **2**:57-60.

3. European Food Safety Authority: **Reasoned opinion on the modification of the existing MRLs for pyriproxyfen in stone fruits and tea**. *EFSA Journal* 2013, **11**(11):3489.

4. Farouket M, Lobna H, El A, Noha F.: **New HPLC and fluorometric methods for the determination of pyriproxyfen and pyridalyl insecticide residues in tomatoes**. *Journal of AOAC International* 2014, **97**(1):188-196.

5. Fathulla R. N, Malson T, Zhao X, Conteh A, Ross L. A, Keller D. L.: **Anaerobic aquatic metabolism of 14C-pyriproxyfen**. *Study HLA 6311-128. Hazleton Wisconsin, Inc, USA* 1995, Report NNM-51-0033. Sumitomo, Japan.

6. Gang L, Xiaojia H, Junde W.: **Quantitative analysis of pyriproxyfen by HPLC**. *Pesticides* 2003, 2003-08.

7. Itoh T, Kawada H, Abe A, Eshita Y, Rongsriyam Y, Igarshi A.: **Utilization of blood-fed females of *Aedes aegypti* as a vehicle for the transfer of the insect growth regulator pyriproxyfen to larval habitats**. *Journal of the American Mosquito Control Association* 1994, **10**:344–347.

8. Loh P, Yap H.: **Laboratory studies of the efficacy and sublethal effects of an insect**

growth regulator, pyriproxyfen (S-3 l 183) against *Aedes aegypti*. *Tropical Biomedicine* 1989, **6**: 7-12.

9. Pengqiang D**,** Xingang L**,** Xiaojun G**,** Fengshou D**,** Jun X**,** Zhiqiang K**,** Yanbin W**,** Yulong Z**,** Yuanbo L**,** Yongquan Z.: **Rapid residue analysis of pyriproxyfen, avermectins and diflubenzuron in mushrooms and their substrate by ultra-performance liquid chromatography coupled with tandem mass spectrometry**, Analytical Methods *2013, DOI: 10.1039/C3AY41074A.*

10. SANCO: ***Method validation and quality control procedures for pesticide residues analysis in food and feed.*** *SANCO/12495/2011*.

11. Sathi T, Tsuda Y, Somboon P, Kawada H, Tagaki M.: **Difference in the larval susceptibility to pyriproxyfen in nine colonies of six vector mosquito species**. *Medical Entomology and Zoology* 2003, **54**: 155-160.

12. Shankar Suman D, Wang Y, Dong L, Gaugler R.: **Effects of Larval Habitat Substrate on Pyriproxyfen Efficacy Against *Aedes albopictus* (Diptera: Culicidae)**. *Journal of Medical Entomology* 2013, **50**(6): 1261-1266.

13. World Health Organization: **Pyriproxyfen in Drinking Water. Background Document for Development of WHO Guidelines for Drinking-Water Quality** (WHO, Geneva), 2004.

14. World Health Organization: **Pyriproxyfen in drinking-water.** *2008, WHO/HSE/AMR/08.03/10.*

15. Zhao G, GUO D, Zhao S, Sheng Y, DENG X.: **Determination of pyriproxyfen residue in vegetables and fruits by liquid chromatography-tandem mass spectrometry,** Chinese Journal of Chromatography *2009, 04.*