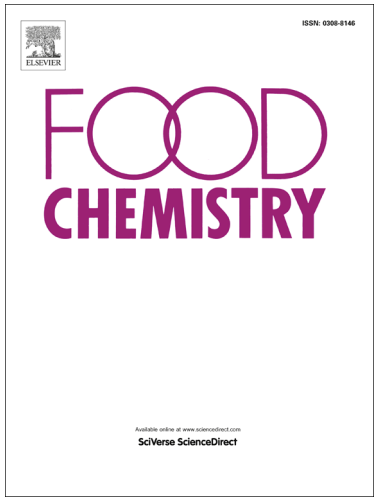
Journal Pre-proofs




# Development and validation of two robust simple chromatographic methods for estimation of tomatoes specific pesticides' residues for safety monitoring prior to food processing line and evaluation of local samples

Amira M. Hegazy, Rehab M. Abdelfatah, Hamada M. Mahmoud, Mohamed A. Elsayed

PII: S0308-8146(19)31766-2
DOI: https://doi.org/10.1016/j.foodchem.2019.125640
Reference: FOCH 125640

To appear in: *Food Chemistry*

Received Date: 25 April 2019
Revised Date: 5 July 2019
Accepted Date: 1 October 2019

Please cite this article as: Hegazy, A.M., Abdelfatah, R.M., Mahmoud, H.M., Elsayed, M.A., Development and validation of two robust simple chromatographic methods for estimation of tomatoes specific pesticides' residues for safety monitoring prior to food processing line and evaluation of local samples, *Food Chemistry* (2019), doi: https://doi.org/10.1016/j.foodchem.2019.125640

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2019 Published by Elsevier Ltd.

# Development and validation of two robust simple chromatographic methods for estimation of tomatoes specific pesticides' residues for safety monitoring prior to food processing line and evaluation of local samples

**Amira M. Hegazy[a*], Rehab M. Abdelfatah[a], Hamada M. Mahmoud[b,c] and Mohamed A. Elsayed[d].**

[a] Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt.

[b] Environmental Sciences and Industrial Development Department, Faculty of Postgraduate Studies for Advanced Sciences (PSAS), Beni-Suef University, Beni-Suef, Egypt.

[c] Zoology Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt.

[d] Analytical Chemistry Department, Faculty of Pharmacy, Fayoum University, Fayoum, Egypt.

***Corresponding author information:**

Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt.

***Tel:*** *+2/ 01013518060* ***Fax:*** *+2/+082/2317950*

***E-mail address****: amira.hegazy@pharm.bsu.edu.eg (*Amira M. Hegazy*)*

Journal Pre-proofs

## ABSTRACT

Combination of pesticides; acetamiprid, flutolanil and etofenprox are usually used for tomato fruits for protecting them against pest infection. Generally, pesticides, residues could be one of the health hazard sources. Two specific simple sensitive chromatographic methods are developed for simultaneous estimation of the concerning pesticides' residues using simple economic steps of field sample preparation. The first method is HP- TLC method. Hexane: methanol: acetone: glacial acetic acid (8: 2: 0.5: 0.1, by volume) is proposed as a developing system. The second one is RP- HPLC. Acetonitrile: water (75: 25, v/v) is proposed as a mobile phase. The recommended methods are completely validated regarding ICH guidelines. Their means percentages and standard deviations of accuracy range 100.32± 0.89- 99.27± 0.9. The methods' repeatability and intermediate precision relative standard deviation percentages range 0.395 & 0.894. They are successfully applied for estimating the pesticides in pure and commercial forms and field samples.



**Keywords:**

Acetamiprid; flutolanil; etofenprox; HP-TLC; RP- HPLC; validation.


Journal Pre-proofs

## 1. Introduction

Many plants' pests and diseases affect food crops. So farmers use the crop specific pesticides to produce abundant high-quality crops (Knowles, 2008), (Garau, Angioni, Aguilera Del Real, Russo, & Cabras, 2002). Tomato fruits have a high anti-oxidant capacity which reduces the risk of cardiovascular and cancer diseases (Raiola, Rigano, Calafiore, Frusciante, Barone, 2014), (Bhuvaneswari & Nagini, 2005) and contain a variety of vitamins and minerals. Fresh tomato fruits could be a potential source of harmful and toxic pesticide residues (Zork & Maja, 2009). At Egypt, Al-Fayoum governorate, location of the study, tomato fruits are sprayed with three pesticides acetamiprid (ACP), flutolanil (FLL) and etofenprox (ETFP), concurrently, to control attack of insects and fungal growth (Approved Recommendations for the Control of Agricultural Pets, Agricultural Pesticide Committee, Egypt, 2015).

Acetamiprid (ACP) is N-[(6-chloropyridin-3-yl) methyl]-N'-cyano-N-methylethanimidamide and has PubChem CID 213021, MF: $C_{10}H_{11}ClN_4$ and MW: 222.676g/mol. Flutolanil (FLL) is N-(3-propan-2-yloxyphenyl)-2-(trifluoromethyl) benzamide and has PubChem CID 47898, MF: $C_{17}H_{16}F_3NO_2$ and MW: 323.315g/mol. Etofenprox (ETFP) is 1-ethoxy-4-[2-methyl-1-[(3-phenoxyphenyl) methoxy] propan-2-yl] benzene and has PubChem CID: 71245, MF: $C_{25}H_{28}O_3$ and MW: 376.496g/mol. The chemical structures of the compounds are figured out in **Figure S1**. They are commonly freely soluble in acetonitrile and methanol (Turner, 2017).

The literature survey shows some published methods which determine them individually; spectrophotometric methods for determination of ACP and FLL residues (Valéria, Sanja, Nataša & Zsigmond, 2012), (Qin, Shi, Gen-di, Hongboand, & Xiao, 2011), (Gallart, Armenta & Guardia, 2016), HPLC methods for determination of ACP and FLL residues residue (Lazić, Šunjka, Grahovac, Guzsvány, Bagi, & Budakov, 2012), (Martı´nez, Gil Garcı´a, Martinez, & Lopez, 2002), (Kikuchi, Sakai, Nemoto, & Akiyama, 2018) and HPLC methods for determination of ETFP residue on vegetables (Jia, Bi, Wang, Qiu, Zhou, & Zhou, 2006), (Sung, Abd El-Aty, Young, Myeong, Mi, & Han, 2014), (Deuk, Hyeok, & Kwon, 2011). In spite of (Hegazy, Abdelfatah, Mahmoud & Elsayed, 2018) has published an HPLC method for determining a limited number of pesticides, they determine pesticides mixture which is different from the concerning mixture. Also, the method is applied to another plant species.

The trend to analyze a narrow range of pesticides belong certain plant product via specific method has been noticed last years (Abdelfatah, Hegazy, Mahmoud & Elsayed, 2018), (Tiwari, & Asthana, 2012), (Baig, Akhtera, Ashfaq and Asi, 2009). A GC-Mass method is found for the estimation of 186 pesticides including the concerned pesticides (Zhao, 2014). The work aims to estimate the residues of tomatoes crop specific pesticides on the fruits before processing and serving stage.

## 2. Materials and methods

### *2.1 Instruments*

*2.1.1 HP-TLC method*

CAMAG TLC scanner operated by winCATS software, HP-TLC 20 x 20 cm aluminum plates coated with 0.25 mm silica gel 60 $F_{254}$ (Merck, Germany), rotary flash evaporator (Shanghai Shensheng Biotech limited Co., China), and TV ultra-sonication (Sonix, USA) were used.

*2.1.2 RP-HPLC method*

1200 infinity series LC connected to 1260 UV - VIS detector with 1260 infinity, and 15 cm x 4.6 mm (i.d 5 µm particle size) Zobrax SB $C_8$ column (Agilent, USA) were used.

***2.2. Pure samples:***

ACP (99.21%), FLL (99.08%) and ETFP (99.18%) were purchased from Sigma-Aldrich (Cairo, Egypt).

***2.3 Commercial formulations:***

- Jinx®(ACP; 20 % w/w, Batch No. 309872), Moncut® (FLL; 25 % w/w, Batch No. 206214) and Primo® (ETFP; 10 % w/w, Batch No. 2046) were purchased from producing companies; Claire Manufacturing Co. (USA), Nichino Inc. (USA) and Shoura Chemicals (Alexandria, Egypt), respectively.

***2.4 Chemicals and reagents:***

Methanol and acetonitrile (HPLC grade) were purchased from Sigma-Aldrich Chemie (GmbH, Germany). Hexane, glacial acetic acid, acetone and sodium sulfate of analytical grade were purchased from El-Nasr Pharmaceutical Chemicals Co. (Cairo, Egypt). De-ionized water was purchased from SEDICO Pharmaceuticals Co. (Giza, Egypt).

***2.5 Standard solutions***

ACP, FLL and ETFP standard stock solution (1000 µg $mL^{-1}$) and standard working solutions (100 & 10 µg $mL^{-1}$) were prepared in methanol.

ACP, FLL and ETFP commercial stock solutions (1000 µg $mL^{-1}$) and working solutions (100 & 10 µg $mL^{-1}$) were prepared in methanol.

### *2.6 Field sampling:*

*2.6.1 Sample preparation:*

Tomato samples were collected from El-azab district, Al-Fayoum Governorate, Egypt during the winter season (average temperature: 20°C). Samples of three kg of previously sprayed ripe tomatoes by the studied pesticides were collected at three replicates each one was one kg so the total weight for all the samples was three kg. The samples were taken 1, 3, 7 and 10 days after spraying by pesticides regarding FAO/WHO guidelines (FAO/WHO. 1985).

*2.6.2 Sample extraction:*

1 Kg of sprayed tomato fruits was peeled then the peels were chopped and shake with 20 mL of acetonitrile for 2 minutes in a 50 ml centrifuge tube then 5 g of sodium sulfate were added. The tube was centrifuged for 2 minutes at 6000 rpm. Supernatant extracts were collected, quantitatively, and concentrated to 3 mL under vacuum using a rotary flash evaporator at 28 °C. All the process of preparation was done thoroughly using clean tools to prevent cross-contamination. Samples were kept at a fridge to minimize pesticides losses via evaporation or degradation according to a recommendation of the European Commission.

### *2.7 Calibration curves*

*2.7.1 HP-TLC method*

A series of serial dilutions of ACP, FLL, and ETFP in concentrations ranges from 0.10-1.40, 0.01-0.22 & 0.01-0.20 µg $mL^{-1}$, respectively, were prepared. Samples were applied on HP-TLC plates using 10 µL of each prepared solution using a Camag-Linomat IV applicator.

*2.7.2 RP-HPLC method*

Different series of serial dilutions of ACP, FLL, and ETFP in concentrations ranges from 1.00-14.00, 0.10-2.20 & 0.10-2.00 µg $mL^{-1}$, respectively, were prepared. Triplicate injections were carried out with injection volume 20 µL.

## 3. Results and discussion

### *3.1-Method development and optimization*

*3.1.1 HP-TLC method*

Chloroform/acetone, hexane/acetone, methanol /chloroform and methanol/hexane were tried but give bad separation. The addition of acetic acid to methanol/ hexane reduces tailing and achieves moderate separation (Benger, 1991). Finally, the developing system methanol: hexane: acetone: glacial acetic acid (8: 2: 0.5: 0.1, by volume) gives optimum separation. $R_f$ values of the concerning pesticides are 0.30, 0.45 and 0.71 for ACP, FLL and ETFP, respectively, **Figure 1**.

*3.1.2 RP-HPLC method*

Multiple chromatographic conditions have manipulated to achieve the best resolution for the three concerning pesticides including organic modifier, scanning wavelength of detection and flow rate. Methanol: water (70: 30 and 50: 50, v/v) and methanol: 0.2 % acetic acid solution (80: 20, v/v) give

bad separation, then acetonitrile: water (75: 25, v/v) gives moderate separation with broad peaks, finally acetonitrile: water (75: 25,v/v), pH adjusted to 2.87 with glacial acetic acid results in the best resolution with no tailing (Hajimehdipoor, Shekarchi, Hamedani, Abedi, Zahedi, & Gohari, 2011). Scanning at 225 nm gives highly sensitive results with the lowest LOD and LOQ values. Many flow rates were tried; 0.60 mL $min^{-1}$ gives the best resolution and short time of estimation. The retention time values are 5.68, 7.50 and 9.01min for ACP, FLL and ETFP respectively, as illustrated in **Figure 2**. By plotting the concentrations versus the relative peak areas, a linear relationship has obtained. **Table I** shows the regression equation parameters.

### *3.2. Method validation*

Method validation has carried out for both proposed methods regarding ICH guidelines (International Conference on Harmonization, 2005).

*3.2.1 Linearity:*

A linear relationship has obtained for both methods in concentration range 0.10-1.20, 0.02-0.25, and 0.02-0.20 µg $band^{-1}$ for each of ACP, FLL, and ETFP by HP-TLC, respectively, and 1.00-14.00, 0.10-2.20, and 0.10-2.00 µg $mL^{-1}$ for each of ACP, FLL, and ETFP by RP-HPLC, respectively, **Table I.**

*3.2.2 Accuracy:*

The recovery % of blind pure pesticide samples have calculated and the concentration ranges have determined using Beer's law, **Table I**. The obtained recoveries percent are 99.24, 99.18, and 99.72 for each of ACP,

FLL, and ETFP by HP-TLC, respectively, and 99.48, 100.09, and 99.68 for each of ACP, FLL, and ETFP by RP-HPLC, respectively, **Table II**.

*3.2.3 Precision:*

It has tested regarding to both repeatability and intermediate precision. Values verify the precision of the proposed methods showing repeatability 0.486, 0.515, and 0.484 for each of ACP, FLL, and ETFP by HP-TLC, respectively, and 0.418, 0.427, and 0.395 for each of ACP, FLL, and ETFP by RP-HPLC, respectively, **Table I**.

*3.2.4 Specificity:*

**Figures 1 & 2** show peaks of the studied pesticides verifying the specificity of the proposed HP-TLC and RP-HPLC methods, respectively, without interference from other excipients. $R_f$ values of the concerning pesticides are 0.30, 0.45, and 0.71 for ACP, FLL, and ETFP, respectively. The retention time ($t_R$) values are 5.68, 7.50, and 9.01min for ACP, FLL, and ETFP respectively. **Table II** shows the values of R% of each drug determined by each method.

*3.2.5 Limit of detection (LOD) and Limit of quantitation (LOQ)*

The limit of detection (LOD) has calculated and found to be 0.030, 0.006, and 0.006 for each of ACP, FLL, and ETFP by HP-TLC, respectively, and 0.031, 0.030, and 0.031 for each of ACP, FLL, and ETFP by RP-HPLC, respectively.

Limit of quantitation (LOQ) has calculated and found to be 0.090, 0.019, and 0.018 for each of ACP, FLL, and ETFP by HP-TLC, respectively, and 0.910, 0.092, and 0.093 for each of ACP, FLL, and ETFP by RP-HPLC, respectively, **Table I**. LOD & LOQ have calculated according to the

reference; (Shrivastava and Gupta, 2011; LOD=3.3xSD of the response/slope,

LOQ=10xSD of the response/slope.

*3.2.6 Robustness:*

Robustness of the methods have verified by deliberate small changes in the chromatographic conditions (USP, 2011), % RSD of each pesticide has calculated for both methods; results are listed in **Table SI**.

*3.2.7 System Suitability:*

**Table III** shows the system suitability testing parameters of the proposed methods.

***3.3 Application to commercial formulations***

The proposed chromatographic methods have used for the determination of ACP, FLL and ETFP in Jinx® Powder, Moncut® and Primo®, respectively, **Table II**.

***3.4 Field sample estimation***

A clean-up, or dilution step may be necessary to reduce matrix interferences and reduce contamination of the instrument system leading to an improved selectivity and robustness (European Commission, 2017). The novel way of sample preparation presented in this work is correlated to peeling just the exposed surface to sprayed pesticides and only peeled surfaces proceed in field sampling. That saves time and cost of sample preparation. Field samples are collected according to WHO/FDA guidelines.

At first day of sampling, the field samples estimation shows values little over the acceptable values of the maximum residues limits (MRLs) that

obey the recommended dose for human health and vegetable productivity regarding FDA authority (Michael, 2015), (Michael, 2010) and (Michael, 2013). But at tenth day, they shows values much less than that of MRLs, the results are listed in **Table SII**.

### *3.5 Statistical estimation*

The results obtained by the two developed chromatographic methods for the estimation of pure samples of ACP, FLL, and ETFP have statistically compared to those obtained by the reported methods (Lazić, Šunjka, Grahovac, Guzsvány, Bagi, & Budakov, 2012), (Kikuchi, Sakai, Nemoto, & Akiyama, 2018), (Sung, Abd El-Aty, Young, Myeong, Mi, & Han, 2014); where no significant differences have found between them, **Table IV**.

## Conclusion

This work presents two highly specific chromatographic HP-TLC and RP-HPLC methods. They could determine the concerning pesticide residues in very low concentrations with perfect accuracy and precision. The novelty of the proposed methods is hidden in determination specifically the pesticides which selectively are used effectively on only palatable tomatoes' parts. The proposed methods, superiorly are more specific, more economic and need less sophisticated tools and follow simpler extraction procedures than the published GC-Mass method (Zhao, 2014). Whereas the publisher uses complicated procedures for sample preparation and using the whole plant consuming longer time and requiring more expensive instrumentation. The most advantage of the methods presented in this work

over the published ones is their cost-effectiveness and simplicity of instrumentation making them suit inspection organizations in developing countries. Thus the proposed chromatographic methods can be used for monitoring ACP, FLL, and ETFP residues on tomato fruits for inspection purposes at Health ministry laboratories. Moreover, they could analyze corresponding commercial formulations without interference from excipients.

***Acknowledgment***

The senior author is appreciated and gives all thanks to Prof. Assis. Khaled A. M. Khaled, Faculty of Agriculture, Beni-suef University, for his generous consultation through writing this work. The authors would like to express her sincere thanks to the Faculty of Pharmacy, Fayoum University for its financial support.



Journal Pre-proofs

279 ***Reference:***

280 Abdelfatah, R.M., Hegazy, A.M., Mahmoud, H.M. & Elsayed, M.A.
281 (2018). Two chromatographic methods for the quantitative determination of
282 some pesticides applied for cucumber pests in Egypt. *Separation Science*
283 *Plus.* 1, 5, 334-342.

284 Approved Recommendations for the Control of Agricultural Pets,
285 Agricultural Pesticide Committee, Egypt, 2015, ISBN 978-977-302-448-2.

286 Baig, S.A. & Akhtera, N.A. & Ashfaq, Muhammad & Asi, M.R.. (2009).
287 Determination of the organophosphorus pesticide in vegetables by high-
288 performance liquid chromatography. American-Eurasian Journal of
289 Agriculture and Environmental Science. 6. 513-519.

290 Benger, B. (1991). Proceeding of the sixth international symposium on
291 instrumental planar chromatography. *Institute for Chromatography*, Bad
292 Duerkheim, 291.

293 Bhuvaneswari, V. & Nagini,` S. (2005). Lycopene: a review of its potential
294 as an anticancer agent, *Curr Med Chem Anticancer Agents*., 5, 627-35.

295 Deuk, Y., Hyeok, C. & Kwon, K. (2011). Liquid chromatographic
296 determination of etofenprox residues in foods with mass-spectrometric
297 confirmation, *Korean Journal of Environmental Agriculture*, 30, 432-439.

298 European Commission, 2017. SANTE / 11813/2017 Guidance document on
299 analytical quality control and method validation procedures for pesticide
300 residues and analysis in food and feed.

301 FAO/WHO. (1985). 2nd edition, Recommended methods of sampling for
302 determination of pesticide residues. *US FDA Federal Register*, 5-25.

303 Gallart, D., Armenta, S. & Guardia, M. (2016). Indoor and outdoor
304 determination of pesticides in air by ion mobility spectrometry. *Talanta*,
305 161, 632-639.
306 Garau, V.L., Angioni, A., Aguilera Del Real, A., Russo, M. & Cabras, P.
307 (2002). Disappearance of azoxystrobin, pyrimethanil, cyprodinil, and
308 fludioxonil on tomatoes in a greenhouse. *Journal of Agricultural and Food*
309 *Chemistry*, 50, 1929-1932.

310 Hajimehdipoor, H. Shekarchi, M., Hamedani, M.P., Abedi, Z., Zahedi, H.
311 & Gohari, A.R. (2011). A validated high performance liquid
312 chromatography method for the estimation of thymol and carvacrol
313 in Thymus vulgaris L. volatile oil. *Iranian Journal of Pharmaceutical*
314 *Research*, 10, 705-710.
315 Hegazy, A.M., Abdelfatah, R.M., Mahmoud, H.M. & Elsayed, M.A.
316 (2018). Two spectrophotometric methods for quantitative determination of
317 some pesticides applied for cucumber in Egypt. *Beni-Suef University*
318 *Journal of Basic and Applied Sciences.*7, 4, 12, 598-605.
319 International Conference on Harmonization (ICH). (2005). Q2R1:
320 Validation of analytical procedures: methodology. *US FDA Federal*
321 *Register*, 65-74.
322 Jia, G., Bi, C., Wang, Q., Qiu, J., Zhou, W. & Zhou, Z. (2006).
323 Determination of etofenprox in environmental samples by HPLC after
324 anionic surfactant micelle-mediated extraction. *Analytical and*
325 *Bioanalytical Chemistry*, 384, 1423-1427.
326 Kikuchi, H., Sakai, T., Nemoto, S. & Akiyama, H. (2018). Total
327 determination of residual flutolanil and its metabolites in livestock products

328 and seafood using liquid chromatography-tandem mass spectrometry. *Food*
329 *additives and contaminants. Part A, Chemistry, estimation, control,*
330 *exposure and risk assessment*, 21, 1-9.
331 Knowles, A. (2008). Recent developments of safer formulations of
332 agrochemicals. *Environmentalist*, 28, 35-44.
333 Lazić, S., Šunjka, D., Grahovac, N., Guzsvány, V., Bagi, F. & Budakov, D.
334 (2012). Application of liquid chromatography with diode-array detector for
335 determination of acetamiprid and 6-chloronicotinic acid residues in sweet
336 cherry samples. *Journal Pesticides and Phytomedicine*, 27, 321-329.
337 Martı´nez, J.L., Gil Garcı´a, M.D., Martinez, M. & Lopez, T. (2002).
338 Determination of acetamiprid by hplc-fluorescence with post-column
339 photoderivatization and HPLC-mass selective detection. *Journal of Liquid*
340 *Chromatography & Related Technologies*, 25, 2695-2707.
341 Michael, G. (2010). Flutolanil pesticide tolerance. *Federal Register*, 75,
342 80346-80350.
343 Michael, G. (2013). Etofenprox pesticide tolerance. *Federal Register*, 78,
344 70870-70877.
345 Michael, G. (2015). Acetamiprid pesticide tolerance. *Federal Register*, 80,
346 68772-68778.
347 Qin, X., Shi, D., Gen-di, J., Hongboand, L. & Xiao, Y. (2011).
348 Determination of acetamiprid by a colorimetric method based on the
349 aggregation of gold nanoparticles. *Microchimica Acta*, 173, 323-329.
350 Raiola, A., Rigano, M. M., Calafiore, R., Frusciante, L., Barone, A. (2014).
351 Enhancing the health-promoting effects of tomato fruit for biofortified
352 food. *Mediators Inflamm*. 2014, 139873.

353 Shrivastava, A. & Gupta, V. B. (2011). Methods for the determination of
354 limit of detection and limit of quantitation of the analytical methods. Chron
355 Young Sci. 2:21-5.
356 Sung, K., Abd El-Aty, A. M., Young, J., Myeong, S., Mi, Y. & Han, J.
357 (2014). A simple HPLC-UVD method for detection of etofenprox in green
358 tea using sample hydration. *Food Science and Biotechnology*, 23, 2097-
359 2101.
360 The United States pharmacopeia. (2011). 34th edn. USA: National
361 Formulary 29, United States pharmacoprial convention INC.
362 Tiwari, N. & Asthana, A. A. (2012). Catalytic Kinetic Spectrophotometric
363 Determination of Organophosphorus Pesticides in Vegetable Samples.
364 *Journal of Brazilian Chemical Socity,* 23, 2, 322-327.
365 Turner, J. A. (2017). The pesticides manual, 17th edition, *British Crop*
366 *Production Council*, 16- 34.
367 Valéria, J., Sanja, L., Nataša, D, V. & Zsigmond, J. (2012). Derivative
368 spectrophotometric determination of acetamiprid in the presence of 6-
369 chloronicotinic acid. *Journal of Serbian Chemical Society*, 77, 911-917.
370 Zhao, P., Huang, B., Li, Y., Han, Y., Zou, N., Gu, K., Li, X. & Pan, C.
371 (2014). Rapid multiplug filtration cleanup with multiple-walled carbon
372 nanotubes and gas chromatography–triple-quadruple mass spectrometry
373 detection for 186 pesticide residues in tomato and tomato products. *Journal*
374 *of Agricultural and Food Chemistry*, 62, 3710-3725.
375 Zorka, K. & Maja S. (2009). Screening of fresh fruit and vegetables for
376 pesticide residues on Croatian market. *Food Control*, 20, 4, 419-422.
377

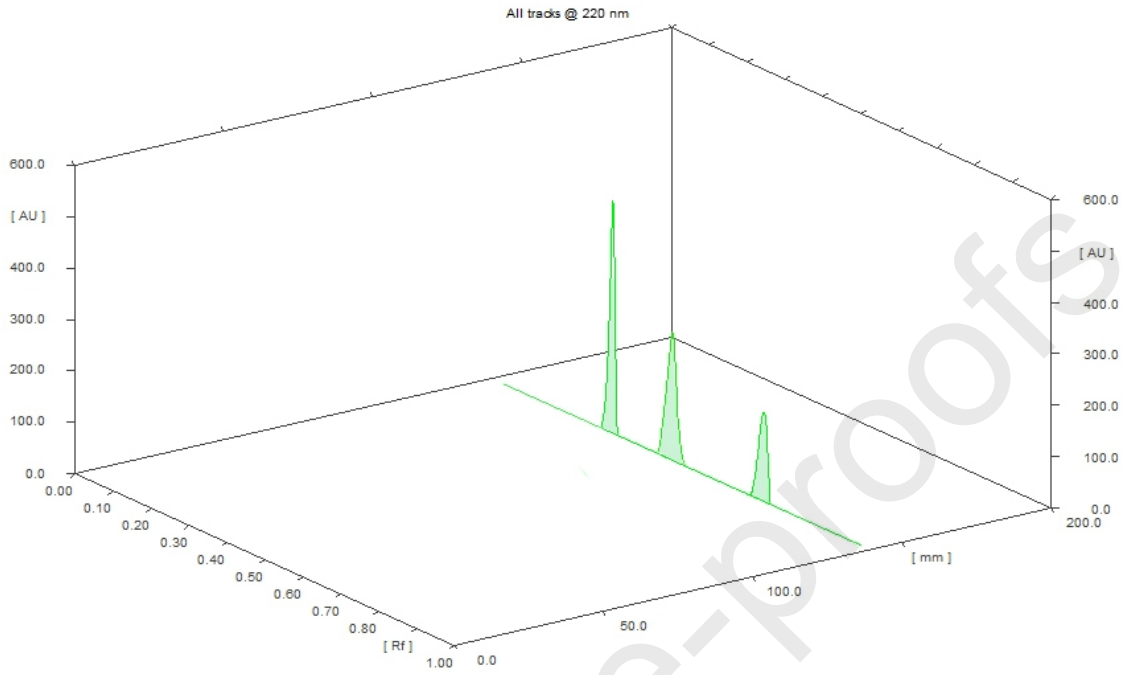
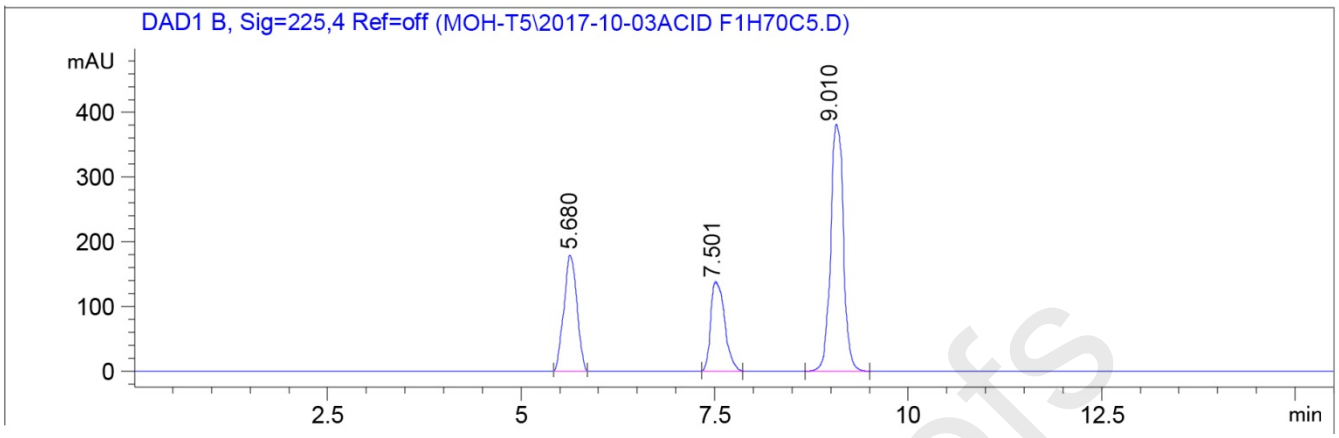



Figure 1: 3D HPTLC-densitogram of a resolved mixture of Acetamiprid ($R_F$=0.30), Flutolanil ($R_F$=0.45) and Etofenprox ($R_F$=0.71) using hexane: methanol: acetone: glacial acetic acid (8: 2: 0.5: 0.1, v/v) as a developing system at 220 nm.

Journal Pre-proofs

393




394

395 Figure 2: HPLC chromatogram of a resolved mixture of Acetamiprid, Flutolanil and
396 Etofenprox using acetonitrile: water (75: 25, v/v), pH adjusted to 2.87 with glacial
397 acetic acid.

398

Journal Pre-proofs

400 Table I: Results of assay validation parameters of the proposed methods; HP-TLC and
401 RP-HPLC.

| **Parameter** | **HP-TLC method** | | | **RP-HPLC method** | | |
|---|---|---|---|---|---|---|
| | ACP | FLL | ETFP | ACP | FLL | ETFP |
| Range | 0.10- 1.2 µg per band | 0.02- 0.25 µg per band | 0.02- 0.20 µg per band | 1.0- 14 µg $mL^{-1}$ | 0.10- 2.5 µg $mL^{-1}$ | 0.10- 2.0 µg $mL^{-1}$ |
| Slope | 0.7956 | 4.4817 | 2.4624 | 0.5009 | 3.9941 | 6.9782 |
| Intercept | 0.0032 | 0.0023 | 0.0068 | 0.0548 | 0.0437 | 0.0341 |
| Correlation coefficient | 0.9999 | 0.9999 | 0.9999 | 0.9999 | 0.9998 | 0.9999 |
| Accuracy (Mean ± SD) | 100.18 ±0.93 | 99.32 ±0.84 | 99.27 ±0.91 | 100.32 ±0.89 | 99.28 ±0.91 | 99.76 ±0.99 |
| **Precision** | | | | | | |
| Repeatability (RSD %) | 0.486 | 0.515 | 0.484 | 0.418 | 0.427 | 0.395 |
| Intermediate precision (RSD %) | 0.802 | 0.894 | 0.704 | 0.813 | 0.795 | 0.687 |
| LOD | 0.030 | 0.006 | 0.006 | 0.031 | 0.030 | 0.031 |
| LOQ | 0.090 | 0.019 | 0.018 | 0.910 | 0.092 | 0.093 |

402

403

404 * The intraday precision (n =3), average of three different concentrations (0.2, 0.6 and
405 0.8 µg $band^{-1}$) of ACP and (0.04, 0.08 and 0.1µg $band^{-1}$) of each of FLL and ETFP for
406 HPTLC method, and (2, 4 and 8 µg $mL^{-1}$) of ACP and (0.2, 0.6 and 1 µg $mL^{-1}$) of
407 FLL and ETFP for RP-HPLC method repeated three times within day. The inter-day
408 precision (n =3) repeated three times in three successive days.

409 **Limit of detection and limit of quantitation are determined via calculations
410 (LOD=3.3xSD of the response/slope, LOQ=10xSD of the response/slope),
411 (Shrivastava & Gupta, 2011).

Journal Pre-proofs

412 Table II: Results of application of the proposed methods to Jinx® Powder, Moncut®

| HP-TLC method | | | | | RP-HPLC method | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Taken | Found* % | Pure added (µg/band) | Pure Found (µg/band) | Recovery % | Taken (µg mL$^{-1}$) | Found* % | Pure added (µg mL$^{-1}$) | Pure Found (µg/band) | Recovery % |
| ACP | ACP | ACP | ACP | ACP | ACP | ACP | ACP | ACP | ACP |
| 0.60 | 99.24 | 0.40 | 0.40 | 99.64 | 5.00 | 99.48 | 3.00 | 3.03 | 101.16 |
| | | 0.60 | 0.61 | 100.85 | | | 5.00 | 5.00 | 99.98 |
| | | 0.80 | 0.79 | 99.07 | | | 7.00 | 7.01 | 100.08 |
| | | Mean± SD | | 99.85±0.91 | | | Mean± SD | | 100.41±0.66 |
| FLL | FLL | FLL | FLL | FLL | FLL | FLL | FLL | FLL | FLL |
| 0.10 | 99.18 | 0.06 | 0.06 | 100.41 | 0.50 | 100.09 | 0.30 | 0.30 | 99.96 |
| | | 0.10 | 0.10 | 98.60 | | | 0.50 | 0.50 | 100.50 |
| | | 0.15 | 0.15 | 100.32 | | | 1.00 | 1.00 | 99.52 |
| | | Mean± SD | | 99.78±1.02 | | | Mean± SD | | 99.99±0.49 |
| ETFP | ETFP | ETFP | ETFP | ETFP | ETFP | ETFP | ETFP | ETFP | ETFP |
| 0.10 | 99.72 | 0.08 | 0.08 | 99.69 | 0.50 | 99.68 | 0.30 | 0.30 | 100.85 |
| | | 0.10 | 0.10 | 101.28 | | | 0.50 | 0.50 | 99.31 |
| | | 0.15 | 0.15 | 99.73 | | | 1.00 | 1.01 | 101.17 |
| | | Mean± SD | | 100.23±0.91 | | | Mean± SD | | 100.44±0.99 |

413 and Primo® and results of application of the standard addition technique.

414

415

416

417 *Average of three determinations.



Journal Pre-proofs

418 Table III: System suitability testing parameters of the proposed methods.

| Parameter | HP-TLC method | | | RP-HPLC method | | | Reference value |
|---|---|---|---|---|---|---|---|
| | ACP | FLL | ETFP | ACP | FLL | ETFP | |
| Tailing factor* (T) | 0.89 | 0.93 | 1.07 | 0.92 | 0.88 | 0.99 | **< 1.5** |
| Capacity factor (K') | 3.10 | 4.62 | 7.21 | 4.51 | 6.52 | 8.01 | **1- 10** |
| Resolution ($R_S$) | 1.69 | | 2.53 | 2.92 | | 2.50 | **> 1.5** |
| Selectivity (α) | 1.53 | | 1.52 | 1.44 | | 1.23 | **> 1** |
| Column efficiency (N) | - | - | - | 1111 | 1600 | 4096 | **Increase with efficiency of the separation** |
| HETP** (cm plate$^{-1}$) | - | - | - | 0.013 | 0.009 | 0.004 | **The smaller the value the higher the column efficiency** |

419 *Calculated using three peaks.

420 **HETP = height equivalent to theoretical plate, (cm plate$^{-1}$).

421

422

423

424

425

426

427

428

429

430

Journal Pre-proofs

Table IV: Statistical comparison of the results obtained by the proposed methods and the reported methods, (Lazić, Šunjka, Grahovac, Guzsvány, Bagi, & Budakov, 2012), (Kikuchi, Sakai, Nemoto, & Akiyama, 2018) and (Sung, Abd El-Aty, Young, Myeong, Mi, & Han, 2014) for determination of the concerning pure pesticides.

| **Parameter** | **HPTLC method** | | | **HPLC method** | | | **Reported methods *** | | |
|---|---|---|---|---|---|---|---|---|---|
| | ACP | FLL | ETFP | ACP | FLL | ETFP | ACP | FLL | ETFP |
| **Mean** | 99.64 | 99.79 | 99.86 | 99.99 | 99.98 | 99.77 | 99.50 | 99.67 | 100.03 |
| **SD** | 0.90 | 0.99 | 0.97 | 0.98 | 0.97 | 0.91 | 1.19 | 1.16 | 1.25 |
| **Variance** | 0.81 | 0.98 | 0.94 | 0.96 | 0.94 | 0.83 | 1.42 | 1.35 | 1.56 |
| **N** | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| **Student's *t*-test* (2.45)** | 0.26 | 0.22 | 0.29 | 0.85 | 0.57 | 0.44 | ---- | ---- | ---- |
| ***F*- test** (4.28)** | 1.75 | 1.38 | 1.66 | 1.48 | 1.44 | 1.88 | ---- | ---- | ---- |

* ACP was analyzed by using acetonitrile: 0.10 N ammonium chloride (80: 20, v/v) and gradient elution at 230 nm.

* FLL was analyzed by LC/MS/MS.

* ETFP was analyzed by using acetonitrile: water (85: 15, v/v) and 1 mL flow rate at 225 nm.

** Figures in parenthesis are the corresponding tabulated values at p = 0.05.

Journal Pre-proofs

**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Journal Pre-proofs

# Highlights

- The developed chromatographic methods are highly sensitive and specific and have more economic methodology.
- The proposed field sample extraction procedures follows novel simple economic steps.
- The methods performance are good consistency with the corresponding GC method and superiorly simpler than it.
- The methods can be used for monitoring the pesticides residues.

Journal Pre-proofs