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Implementing the contamination prevention programs in the pesticide industry by infrared spectroscopy

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ABSTRACT

An infrared spectroscopy based methodology has been successfully developed to implement contamination prevention programs in the pesticide industry. Sensitivity of the IR procedure, traditionally considered the Achilles Hell of the technique, has been improved by using a transmission cell with an open upper side, an internal volume of 35 μL and an optical pathlength of 0.5 mm, providing detection limits of 32 mg L^{-1} for folpet and 48 mg L^{-1} for cymoxanil. The manufacturing of folpet and cymoxanil was employed as an example and the IR methodology was validated for the implementation of contamination prevention programs in the pesticide industry. The swab test and rinsate method were employed as sampling methods and results obtained by both were compared and correlated. Samples were analyzed from a qualitative and quantitative point of view. Qualitative information can be obtained by comparing the sample spectra with those of a new IR spectral library with approximately 50 entries of pesticide standards. Positive identification of folpet in all the analyzed samples was obtained. Other pesticides present in swab and rinsate samples positively identified by IR and confirmed by gas chromatography–mass spectrometry (GC–MS), were metalaxyl and chlorpyrifos methyl used in the manufacture of previous formulations. The amount of folpet in the swab and rinsate samples obtained by the developed IR method was compared with those of a reference procedure, being statistically comparable.

1. Introduction

The development of appropriate cleaning validation programs is receiving especial attention in different industrial sectors as a part of the quality control guidelines of the manufacturing process. It is particularly true in the pharmaceutical, biotechnological and cosmetic sectors, especially in plants with equipment dedicated to multi-product manufacture or packaging, where it is necessary to validate cleaning procedures because it is a regulatory requirement and it also assures, from an internal control and compliance point of view, the quality of the process [1–3].

Reactors cleaning validation is being gradually incorporated in the pesticide industry where the prevention of the contamination of commercially available pesticide products with residual impurities is an issue of growing concern for pesticide manufacturers, tollers and packagers [4]. It is well known that contamination of a commercial product with impurities of other pesticide can result in adverse effects on sensitive treated crops or non-target species and may trigger regulatory issues. Moreover, those incidents may also damage the reputation of the manufacturer company [3].

The assessment of the cleaning method capability implies the process of providing documented evidences that the cleaning methods employed within a facility consistently controls potential carryover of active products into the subsequent product to a concentration which is below predetermined levels. This process implies four key elements: (i) definition of the correct cleaning levels, (ii) establishment of appropriate manufacture equipment cleaning methods, (iii) development of appropriate analytical methodologies to optimize and validate the cleaning procedures and (iv) correct documentation of the aforementioned elements.

There is a lack of legislation regarding contamination prevention in the pesticide manufacture industry and only the USA Environmental Protection Agency (EPA) published in 1996 a notice addressed to manufacturers, formulators, producers and registrants of pesticide products regarding the maximum toxicologically significant levels of impurities of pesticide active ingredients present in technical grade active ingredients or products produced by an integrated system [5]. In this document, the EPA defined the toxically significant levels of contaminants as a function of the type of contaminant and the type of pesticide that is contaminated, establishing nine categories where the toxicological significant levels range from 1 to 1000 ppm.

Cross contamination in phytosanitary production plants could be an important problem from the environmental or legislative point of view. In a multipurpose non-dedicated production line, they can be

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manufactured pesticide products to be commercialized in specific areas such as the European Union (EU) or the USA together with products to be exported worldwide and that are not approved in EU and USA. So, the presence of residues of not approved active ingredients in pesticide products to be commercialized in a market due to cross contamination during the production step could be a serious problem [3].

Any analytical methodology used in the contamination prevention programs can be divided into two parts, sampling and detection. The most prevalent sampling method is based on the analysis of the last rinsate after having flushed a cleaning medium through the equipment [3]. However, the analysis of the rinsate does not guarantee that the impurities are below the defined level in the succeeding product, especially in the analysis of solid formulations, because previously manufactured products may remain in the equipment in the form of lumps located in dead spaces of the production line and may dislodge during the manufacturing of succeeding products [3]. On the other hand, typical analytical methods for residue analysis include gas chromatography–mass spectrometry (GC–MS) and liquid chromatography (LC) with diode array detection [6] to achieve the selectivity and sensitivity required. In those methods, the time for sample preparation and analysis typically means that results are available between hours or days from the collection of samples. Thus, it implies a considerable effort, in terms of time and money, to appropriately validate a cleaning procedure.

Because of that, in this paper, a fast infrared (IR) spectroscopy based methodology has been developed to implement contamination prevention programs in the pesticide industry. Due to its intrinsic characteristics, IR spectroscopy provides a fast and less expensive alternative to chromatographic procedures that reduces solvent consumption and minimizes waste generation [7]. However, sensitivity has been traditionally considered the Achilles Hell of the technique, and spectroscopists are continuously looking for methods to improve the limits of detection and make possible trace level analysis [8].

The use of transmission measurements with increased optical pathlength cells combined with reduced internal volumes can result in a good choice in order to provide an improved sensitivity of IR measurements, especially in those cases where the solvent used is a chlorinated one, such as chloroform. Thereby, in the present study it has been implemented a transmission cell with an open upper side to improve the sensitivity of the IR control method, providing detection limits of the order of parts per million, without sacrificing the simplicity which could be appropriate for the monitoring of the contamination prevention programs.

The production line selected to implement the contamination prevention program of a pesticide company has been one devoted to the manufacturing of solid products, because a successful cleaning procedure is usually harder to achieve than in the case of liquid formulations. Using the manufacturing of folpet and cymoxanil formulations as example, the methodology was validated and results were compared with those obtained by a LC reference procedure. It should be mentioned that sampling has been performed using the swab methodology and rinsates. The swab is recommended in the cleaning verification programs of the pharmaceutical industry [9] and it was done in different points of the inner surface of the mixers of the production line. Additionally, the rinsate of the manufacturing line was analyzed to find a correlation between both values.

2. Experimental section

2.1. Reagents

Folpet and cymoxanil Pestanal grade standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Kaolin technical grade standard, used as inert material in the pesticide industry and for cleaning the production line, was kindly provided by a Spanish pesticide manufacturing company.

All the solvents used in this study were HPLC grade or higher. Acetonitrile was provided by VWR (Fontenay-sous-bois, France). Methanol was acquired from Panreac (Barcelona, Spain). Ethanol, 2-propanol and chloroform, stabilized with amylene (150 $\mu g\,mL^{-1}$), were purchased from Scharlau Chemie S.A (Barcelona, Spain). Water for the LC analysis, with a maximum resistivity of 18.2 M Ω, was obtained from a Milli-Q Millipore system (Bedford, MA, USA).

Stock solutions of folpet and cymoxanil were prepared in chloroform at a concentration level of 5000 $mg\,L^{-1}$. A calibration line ranging from 25 to 1000 $mg\,L^{-1}$ was prepared by appropriate dilutions of the stock solution in chloroform for IR analysis.

2.2. Infrared spectroscopy

IR spectra were recorded using a Tensor 27 FTIR spectrometer from Bruker (Karlsruhe, Germany) equipped with a DLaTGS detector. Spectra were obtained by coadding 10 scans at a resolution of 4 cm^{-1} and a scanner velocity of 10 kHz HeNe frequency, from 4000 to 800 cm^{-1}. For instrumental and measurement control, spectra treatment and data manipulation, it was employed the OPUS program (version 6.5) from Bruker.

In this study, a transmission cell with an open upper side (see Fig. 1a) has been used to improve the sensitivity of the method without sacrificing the simplicity. Thus, a standard transmission flow cell with 2 mm thick CaF_2 windows has been equipped with two Teflon spacers providing a pathlength of 0.5 mm and an internal volume of approximately 35 μL.

Once the cell was assembled, standard and sample absorbance were measured by transmission mode using manual introduction of solutions inside the cell, using a Hamilton 50 μL syringe (Bonaduz, Switzerland) and chloroform as background. Cleaning of the cell was achieved by three sequential injections of chloroform blank solutions.

2.3. Swab sampling procedure

For swab sampling procedure, TX®715 Large Alpha® Sampling Swab (CleanTips® Swabs) from ITW Texwipe (Kernersville, NC, USA) were used. They are double layer polyester swabs specifically engineered for cleaning validation purposes. The swab handled is notched to snap off the head for convenient sample handling and the heads of the polyesters swabs were thermally bonded to the handles without adhesives, avoiding possible contamination during analyte extraction. The swabs were also laundered by the manufacturer to minimize inherent non volatile residues or particulates that could affect the sensitivity and selectivity of the analysis [10].

To simulate the cleaning validation of manufacture equipment surfaces, polished stainless steel and iron, with different oxidation degrees, plates (5 cm × 5 cm) were used in laboratory recovery studies. 100 μL of folpet-cymoxanil (1:1) stock solutions, corresponding to 30, 35 and 40 $\mu g/25\,cm^2$ area, were directly spiked onto the plates, covering homogeneously the complete surface of the plate and they were allowed to dry in the fume hood. All samples were prepared in triplicate. For recovery experiments, the swabs were wet via their immersion into a 2 mL acetonitrile solution. Swabbing implies a systematic multi-pass of the soaked swab over the defined area. In our case, we used eight side by side strokes vertically, eight horizontally and eight each with the flip side of the swab in each diagonal direction. The soaked swab should be firmly passed and, after that, the swab stem was cut approximately 1 cm above the swab head and transferred to a vial containing 2 mL acetonitrile. The swab extraction procedure was repeated two times and the extraction solutions were mixed, evaporated to dryness, reconstituted in 100 μL chloroform and analyzed by IR.

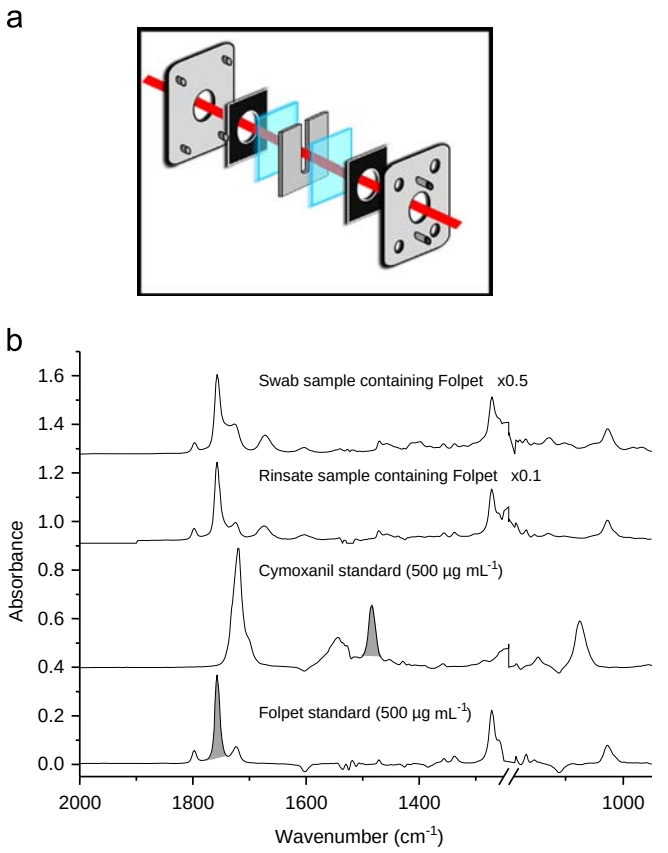


Fig. 1. (a) Transmission cell with an open upper side used in this study and (b) IR absorbance spectra from 2000 to 900 cm^{-1} of folpet and cymoxanil standards and a rinsate and swab sample collected from a production line.

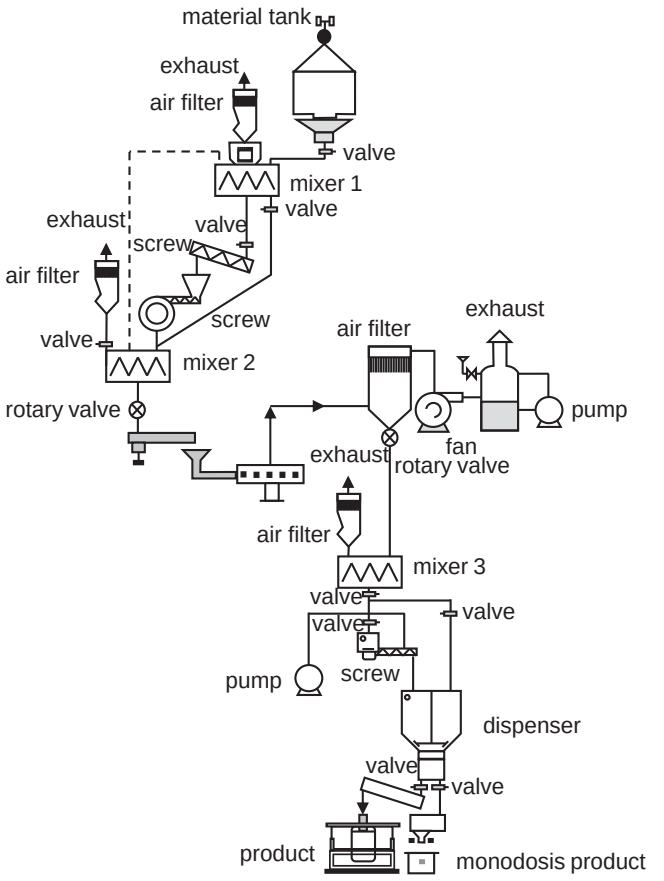


Fig. 2. Production line used to produce solid commercial pesticide formulations in a Spanish company.

Prior to be used for swab sampling, the stainless steel and iron plates were cleaned with isopropanol and acetone and after that the surfaces was sampled with the swabs following the aforementioned procedure and the resulting solution analyzed by IR. In all the cases, the absence of any interferent was assessed.

2.4. Rinse sampling procedure

As it has been aforementioned, the cleaning procedure of the production line includes a flushing step with solid and inert material such as bentonite, kaolin, sand, silica, talc,… or a combination of carrier and surfactants. In this case, 550 kg of a mixture of kaolin and silica (10:1 m/m) were used as solid flushing material.

Approximately 500 g of rinsate were sampled inside the mixers M1, M2 and M3 of Fig. 2 and at the end of the production line. After that, 100 mg of each rinsate sample were accurately weighted inside 10 mL glass vials and pesticides were extracted with 3×1 mL of acetonitrile. The extraction solutions were filtered through a 0.22 µm syringe nylon filter, mixed, evaporated to dryness under a nitrogen flow, reconstituted in an appropriate amount of chloroform and analyzed by IR.

2.5. Reference procedures (see Suplementary material)

2.5.1. Liquid chromatography-diode array detector (LC-DAD)

The LC reference procedure is an adaptation of the AOAC CIPAC method [11]. The separation and detection was performed in an Agilent 1100 Series (Palo Alto, CA, USA) equipped with a Nova-Pack® C-18 4 µm, 3.9×150 mm column, provided by Waters (Milford, Massachusetts, USA) and a diode array detector, working in the absorption range from 200 to 400 nm. The most appropriate wavelength was selected for the determination of both active principles; 254 nm for folpet and 240 nm for cymoxanil. A 10-µL sample or standard volume was directly injected in an isocratic mobile phase consisting of an acetonitrile/water mixture (85:15 v/v) at a flow rate of 1 mL min^{-1}. It should be mentioned that samples were filtered through a 0.22 µm syringe nylon filter prior LC injection.

2.5.2. Gas chromatography–mass spectrometry (GC–MS)

The GC–MS procedure is based on that of Cunha and Fernandes [12]. It was performed with a GC 7890 A from Agilent (Palo alto, CA, USA) equipped with an Agilent HP-5MS capillary column (30 m × 0.30 mm × 0.25 µm) and a Agilent MS 5975C single quadrupole detector.

One microliter of samples was injected in splitless mode at 250 °C employing a constant flow of 1.3 mL min^{-1} of He as a carrier gas. The oven temperature program was 70 °C, increased at 10 °C min^{-1}–130 °C min^{-1}, 6 °C min^{-1}–230 °C and 8 °C min^{-1}–250 °C and held for 13 min. The transfer line and source temperatures were 280 °C and 250 °C, respectively. Electron impact ionization was performed with electron energy of 70 eV and 540 mL min^{-1} helium flow rate as damping gas. Data were acquired in full-scan, from 40 to 650 m/z and in selected ion monitoring (SIM).

3. Results and discussion

3.1. FTIR spectra of folpet and cymoxanil

Folpet, N-[(trichloromethyl)thio]phthalimide, is a protective leaf fungide which inhibits the normal cell division of a broad

spectrum of micro-organisms. Fig. 1b shows the FTIR absorbance spectra in the wavenumber region from 2000 to 900 cm^{-1} of a folpet standard solution prepared in chloroform. As it can be seen the folpet spectrum has three absorption bands, at 1724, 1757 and 1798 cm^{-1}, due to carbonyl in-phase and out-of-phase stretching, a band at 1271 cm^{-1} due to ring stretching of benzene and one at 1026 cm^{-1} due to ring "breathing" [13].

On the other hand, cymoxanil, 2-cyano-N-[(ethylamino) carbonyl]-2-(methoxyimino)acetamide is an acetimide compound used as both, curative and preventative foliar fungicide, on crops including potatoes, tomatoes, and grapes. As it can be seen the cymoxanil spectrum has four main absorption bands, at 1720 cm^{-1}, due to carbonyl stretching, a band at 1543 cm^{-1} due to the NH II band (1st amide), a band at 1483 cm^{-1} due to the NH II band (2nd amide), and one at 1076 cm^{-1} due to the CNH stretching vibration [14].

3.2. Quantitative IR analysis

The performance of the method was evaluated through estimation of the linear range, linearity, precision, and limit of detection (LOD), and results obtained are summarized in Table 1. Quantification was based on the measurement of the band area between 1762 and 1753 cm^{-1} corrected using a two points baseline from 1785 to 1745 cm^{-1} for folpet and the band area between 1491 and 1477 cm^{-1} corrected using a two points baseline from 1502 to 1462 cm^{-1} for cymoxanil. The bands used as analytical response are marked in gray in Fig. 1b.

A six-point calibration curve ranging from 25 to 1000 mg L^{-1} was constructed by appropriate dilutions of the concentrated standard solutions using chloroform for each one of the target analytes. Correlation coefficients, higher than 0.995 were obtained, indicating a linear correspondence between band area and analyte concentration for both studied molecules (see Table 1).

Precision was evaluated as relative standard deviation (RSD) established from five independent measurements of a 50 mg L^{-1} pesticide standard solution, obtaining RSD values lower or equal to 5%. The LOD values were calculated as three times the standard deviation of the intercept divided by the slope of the respective calibration lines. LOD values of 32 and 48 mg L^{-1} were found for folpet and cymoxanil, respectively.

Folpet and cymoxanil are co-formulated in different pesticide products and, thus, mutual interferences should be evaluated. A six-point calibration curve ranging from 25 to 1000 mg L^{-1} was constructed by appropriate dilutions of a concentrated standard solution of folpet:cymoxanil (1:1) using chloroform. The regression coefficients of both calibration lines obtained from the folpet and cymoxanil mixture were compared with those of the calibration lines obtained from the individual standards. Table 1 shows the obtained values of the linear regressions and, as it can be seen, the slope and the intercept of both calibration lines are statistically comparables for a probability level of 95%, and, thus, it can be concluded that there are no appreciable interferences due to the presence of one pesticide in the determination of the other.

Moreover, to avoid the high degree of cross-correlation between the concentration of folpet and cymoxanil obtained in the previous calibration lines, a calibration line of folpet from 25 to 1000 mg L^{-1} was obtained in the presence of a constant concentration of 500 mg L^{-1} of cymoxanil and a calibration line of cymoxanil from 25 to 1000 mg L^{-1} was obtained in the presence of a constant concentration of 500 mg L^{-1} of folpet (see Table 1). As it can be seen, the presence of folpet and cymoxanil at increasing concentrations do not interfere in the determination of the other pesticide, when appropriate measurement conditions are used. Moreover, the slope and the intercept of both calibration lines are statistically comparables with previously reported for a probability level of 95%, and, thus, it can be concluded that there are no appreciable interferences. However, it should be highlighted that the intercept of the calibration line of cymoxanil when folpet is present at 500 mg L^{-1} concentration level is statistically higher than the previous one (0.12 ± 0.02). However, when this value has been interpolated in the calibration line obtained from individual standards of cymoxanil, the concentration obtained (35 mg L^{-1}) is lower than the LOD of the methodology (48 mg L^{-1}).

So, it can be concluded from the previous experiments that the developed methodology is extremely valuable for the simultaneous determination of both pesticides in the same sample, and this residues of folpet and/or cymoxanil can be determined in swabs applied to the manufacturing equipment employed in the phytosanitary production plants and the rinsates obtained after pesticide formulation productions.

3.3. Residues in the manufacture equipment surfaces (swab test)

To perform the manufacture equipment surface recovery experiments, an approach based in the swab test was applied. In this technique a swab was firmly passed through the manufacturing equipment to extract the amount of active ingredients deposited onto the surface. The recovery of the target analyte from the surface, using the swab technique, is mainly affected by the type of swab, the solvents used to moisten the swabs and the times that the swab was passed over the surface.

3.4. Study of extraction conditions

To evaluate the most appropriate conditions for folpet and cymoxanil extraction from equipment surfaces using the swab technique, 100 μL of a 450 mg L^{-1} folpet and cymoxanil mixture (1:1) were spiked onto a 5×5 cm stainless steel plate and then evaporated to dryness to deposit residues corresponding to 45 μg/25 cm^2.

In this study, double layer polyester swabs specifically engineered for cleaning validation purposes were used. Swab sampling is a process that generally comprises several manual steps, and is an inherently subjective activity that varies from operator to operator. It is essential to have a standardized swabbing motion

Table 1
Analytical features of merit of folpet and cymoxanil determination by IR.

Analyte	Interferent	Slope	Intercept	R^2	% RSD[a]	LOD (mg L^{-1})[b]
Folpet	–	0.00262 ± 0.00006	0.05 ± 0.03	0.998	2.7	32
	Cymoxanil (ratio 1:1)	0.00249 ± 0.00005	0.01 ± 0.03	0.998	2.5	34
	Cymoxanil (500 mg L^{-1})	0.00250 ± 0.00009	-0.03 ± 0.05	0.995	2.0	58
Cymoxanil	–	0.00258 ± 0.00009	0.03 ± 0.04	0.996	4.2	48
	Folpet (ratio 1:1)	0.00268 ± 0.00003	-0.05 ± 0.03	0.9990	7.8	22
	Folpet (500 mg L^{-1})	0.00252 ± 0.00004	0.12 ± 0.02	0.9992	6.8	20

[a] % RSD established from four independent measurements of a 50 mg L^{-1} folpet and cymoxanil standard solution.
[b] LOD calculated as three times the standard deviation of the intercept divided by the slope of the respective calibration lines.

to ensure that recoveries could be replicable regardless of who performs the swabbing. In this study we have used the recommended procedure normally employed in the pharmaceutical industry [6].

For analytes extraction, different solvents were evaluated. Results of these experiments are shown in Table 2. As it can be seen, probably due to the low solubility of the active pesticide ingredients in alcohols, the recovery obtained using 2-propanol, ethanol and methanol as extraction solvents was very low. On the other hand, recovery values of 94 ± 4 and $100 \pm 6\%$ for folpet and cymoxanil, respectively, were obtained using acetonitrile as extraction solvent, which implies that the swab technique can be successfully applied for folpet and cymoxanil cleaning verification analysis.

As it has been aforementioned, the extraction solutions were evaporated to dryness and reconstituted in chloroform prior to be analyzed by IR. The effect of the volume of chloroform has been also evaluated from 50 to 100 μL and results are summarized in Table 2. The volume of the IR cell is 35 μL and, thus, a volume of 50 μL of chloroform would be enough to fill the cell obtaining a concentrated solution. However, it can be observed that for a constant amount of folpet and cymoxanil of 45 μg the recoveries obtained using 50, 60 and 80 μL are lower than 85%. It could be due to that using a so small volume of solvent the quantitative recovery of the pesticides from the vials in which sample extracts were evaporated cannot be quantitative, and, that is the reason why the use of a 100 μL of chloroform was selected for further analysis.

3.5. Effect of the material of the manufacturing equipment

Cleaning validation coupons are normally used in the laboratory to evaluate a proposed swabbing method before using that method on the actual manufacture equipment surface, which is the subject of cleaning validation. The cleaning validation coupons should match, as much as possible, the material of construction of the surface of the manufacture equipment. In this study, in order to generalize the applicability of the proposed methodology we have evaluated different coupons constructed with materials normally used in the manufacturing of production machinery. The recoveries of folpet and cymoxanil using the swab test with the previous experimental conditions were evaluated onto polished stainless steel and iron, with different oxidation degrees, coupons (5 cm × 5 cm) (see Table 2).

As it can be seen in Table 2, the material of the equipment drastically affects the recoveries obtained for folpet and cymoxanil.

Table 2
Evaluation of the swab extraction conditions to determine folpet and cymoxanil.

	Folpet recovery (%)	Cymoxanil recovery (%)
Solvent		
2-propanol	48 ± 6	52 ± 3
Ethanol	44 ± 2	66 ± 2
Methanol	39 ± 11	73 ± 9
Acetonitrile	94 ± 4	100 ± 6
Final $CHCl_3$ volume (μL)		
100	93 ± 11	95 ± 9
80	84 ± 2	85 ± 3
60	82 ± 10	82 ± 7
50	77 ± 11	81 ± 2
Material		
Stainless steel	94 ± 4	100 ± 6
Iron	63 ± 2	70 ± 3
Oxidized iron	44 ± 5	45 ± 3
Very oxidized iron	23 ± 4	15 ± 6

Note: Amounts of folpet and cymoxanil of 45 μg were deposited on different surfaces (see text for details).

Moreover, the oxidation degree of iron coupons also affects to the recovery of both pesticides, because oxidation affects the porosity and the rugosity of the material and it could be the reason of the low recoveries obtained when the degree of oxidation of the material increases, being also that the reason for cross-contamination in old mixers due to the retention of previously produced formulations.

3.6. Residues in the rinsate

In contamination prevention programs related to dry (solid) formulations production, the cleaning methods generally involve a step called “flush” cleaning in which a solid, inert material is flushed through the manufacturing line to remove the traces of active ingredient/s previously manufactured. As it has been aforementioned in the introduction section, the most prevalent sampling method employed in the phytosanitary sector is based on the analysis of the last rinsate flushed through the equipment.

To evaluate the capability of the IR methodology developed to determine folpet and cymoxanil in rinsate samples, different samples have been prepared in the laboratory by mixing appropriate amounts of folpet and cymoxanil with kaolin, an inert material normally used in the pesticide industry, to obtain solid samples at concentration levels of 250, 500 and 1000 mg L^{-1}.

Recovery values of 100 ± 3, 98 ± 2 and $100 \pm 2\%$ for 25, 50 and 100 μg folpet, and 100 ± 9, 102.1 ± 0.9 and $99 \pm 2\%$ for 25, 50 and 100 μg cymoxanil, respectively, demonstrate that the developed methodology provides quantitative recoveries for both pesticides at the three concentration levels evaluated and could be used in the implementation of contamination prevention programs in the pesticide industry.

3.7. Analysis of samples after the manufacturing of a formulation

The cleaning procedure used in a Spanish pesticide manufacturing company for cleaning after changeovers in formulation and packaging of solid products included different steps: (i) complete drainage of the installation including manual cleaning of mixers and accessible parts and dry cleaning to remove possible deposits of solids by vacuum cleaning, (ii) flush cleaning with pure solid inert material or a combination of carrier and surfactants according the composition of the preceding product and (iii) complete drainage of the installation and dry cleaning by vacuum aspiration.

Swab sampling was performed in triplicate in five different positions of three horizontal mixers located along the production line, as indicated in Fig. 2 (red points). Sampling was performed before the step (ii) and after the last step (step iii) of the cleaning procedure. Moreover, approximately 500 g of rinsate was sampled inside mixers M1, M2 and M3 of the production system shown in Fig. 2 and at the end of the production line.

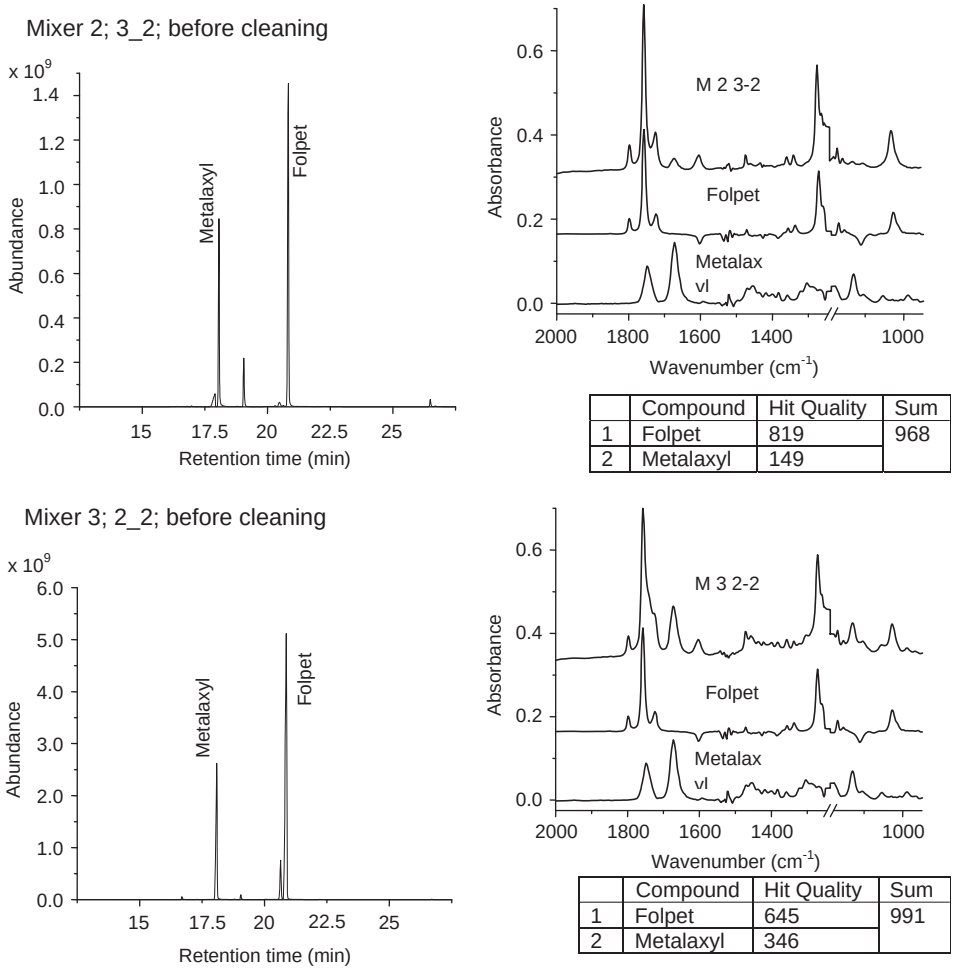
On one hand, a qualitative study of the IR spectra was performed to obtain the maximum information from them. A new IR spectral library, with approximately 50 entries, was created using the OPUS program (version 6.5) from Bruker by recording IR spectra of pesticide standards, including most of those produced in the Spanish company. The software is an easy-to-use and powerful tool which allows comparing an unknown spectrum with the library spectra, detecting and reporting those spectra, from the IR library, which show distinct similarities to the unknown spectrum. The used algorithm calculates the sum of the squared deviations between the query spectrum and the result spectrum for the data points of the wavenumber range defined. In our case, the spectral range selected was between 2000 and 950 cm^{-1}. Spectra were normalized using the minimum–maximum method on the first order derivative spectra. The results were classified according to their similarities using a hit quality parameter that varies from 1000, a perfect conformity, to 0, no correlation at all.

From the IR spectra of samples, it was obtained the positive identification of folpet in all the analyzed samples, swabs and rinsates, (see Fig. 1b as an example) with an average hit quality of 749. Other pesticides present in the swab and rinsate samples positively identified by IR and confirmed by GC–MS, were metalaxyl, average hit quality of the swab samples 236, present in the 86.7% of the samples and average hit quality of the rinsates of 152 and present in the 37.5% of the samples. It should be highlighted that the sum of the average hit quality of folpet and metalaxyl were 978 and 958 for swab and rinsate samples, respectively, indicating that both pesticides were the main components of the mixtures and explain almost all the IR spectra. Moreover, in one sample, coded M 1-2, the pesticide chlorpyrifos methyl was positively identified using the new spectral library in the first place with a hit quality of 254. All the pesticides identified with the spectral IR library were positively confirmed by GC–MS as it can be seen in Figs. 3 and 4.

Moreover, a quantitative analysis of the amount of pesticide residues present in swab and rinsate samples can be easily performed by IR, allowing the evaluation of the pre-established contamination prevention program. Quantitative results obtained for the analysis of rinsate samples and sample swabs are summarized in Table 3. As it can be seen results obtained by IR are statistically comparables to those obtained by the LC reference procedure demonstrating the accuracy of the developed method.

The regression between concentrations found by the reference LC procedure and those obtained by the developed IR method provides an equation with the intercept and slope values statistically comparables to 0 and 1, respectively, for a probability level of 95%, thus indicating that the developed procedure provides acceptable accuracy. It should be highlighted that depending on the concentration of the sample, the final volume of chloroform in which the solid residue was reconstituted varied from 8000 to 100 μL. To illustrate, suppose we analyze a rinsate sample with a concentration of folpet of 1000 mg kg^{-1}, the volume of chloroform to dissolve the residue should be 500 μL to obtain a concentration of folpet in the solution of 200 mg L^{-1}, considering a sample amount of 0.1 g. If the concentration of folpet in the sample is 10,000 mg kg^{-1}, the volume of chloroform to dissolve the residue should be 5000 μL to obtain a concentration of folpet in the solution of 200 mg L^{-1}, considering the same sample amount.

The milligrams per 25 cm^2 of folpet obtained for the mixers 1, 2 and 3 can be converted to milligrams per kilogram taking into consideration the surface of the mixers (without considering the helix), the amount of inert material used in the manufacturing and cleaning step and the state of the surface material. Thus, 353, 97 and 132 mg kg^{-1} folpet would be obtained in the mixer M1, M2 and M3, respectively. Comparing those values with the concentrations obtained in the rinsate samples, in these mixers and at the end of the production line, it seems evident that mixers could be a critical point but there are other important points not considered in the actual cleaning procedure which contribute to increase the cross contamination between manufactured products. Moreover, the concentration of folpet found in the rinsate sample at the end of the production line, encourages us for adding another flush cleaning step to the contamination prevention program before to start the production of other pesticide formulations.



	Compound	Hit Quality	Sum
1	Folpet	819	968
2	Metalaxyl	149	

	Compound	Hit Quality	Sum
1	Folpet	645	991
2	Metalaxyl	346	

Fig. 3. Comparison of GC–MS chromatograms and results obtained from the IR library in the qualitative analysis of swab samples before the cleaning procedure.

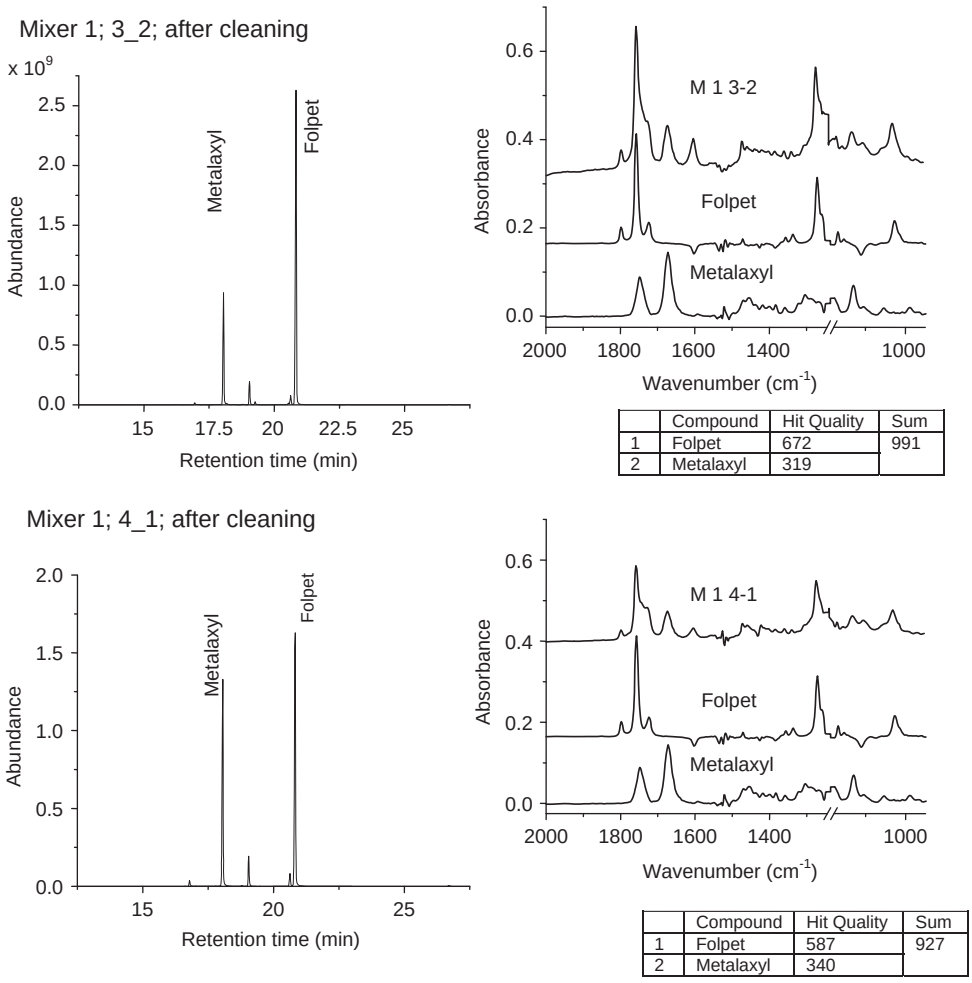


Fig. 4. Comparison of GC–MS chromatograms and results obntained from the IR library in the qualitative analysis of swab samples after the cleaning procedure.

Table 3
Quantitative results obtained for the analysis of sample swabs and rinsate samples by the proposed IR and the reference LC procedures.

	Folpet concentration (mg/25 cm)			
	Before flush cleaning		After flush cleaning	
	IR	LC	IR	LC
Swab samples				
M1 1	4 ± 3	2.54 ± 0.06	0.43 ± 0.02	0.448 ± 0.007
M1 2	17 ± 11	16.17 ± 0.04	0.31 ± 0.03	0.363 ± 0.002
M1 3	2.8 ± 0.3	1.633 ± 0.003	0.44 ± 0.13	0.56 ± 0.02
M1 4	1.1 ± 0.6	1.186 ± 0.011	0.32 ± 0.07	0.3792 ± 0.0004
M1 5	5.5 ± 0.8	2.48 ± 0.03	1.00 ± 0.05	1.101 ± 0.004
M2 1	1.4 ± 0.6	0.87 ± 0.06	0.29 ± 0.10	0.286 ± 0.005
M2 2	1.7 ± 0.5	1.32 ± 0.11	0.55 ± 0.14	0.598 ± 0.004
M2 3	3.4 ± 0.6	1.70 ± 0.09	0.28 ± 0.03	0.270 ± 0.012
M2 4	0.49 ± 0.02	0.623 ± 0.011	0.7 ± 0.5	0.458 ± 0.003
M2 5	2.574 ± 0.006	2.53 ± 0.03	0.41 ± 0.07	0.414 ± 0.005
M3 1	0.67 ± 0.07	1.24 ± 0.04	0.262 ± 0.013	0.258 ± 0.010
M3 2	1.1 ± 0.3	1.005 ± 0.016	0.69 ± 0.07	0.823 ± 0.002
M3 3	1.04 ± 0.04	0.93 ± 0.02	0.60 ± 0.11	0.61 ± 0.7
M3 4	4 ± 2	1.8 ± 0.4	1.7 ± 0.4	1.4 ± 0.2
M3 5	1.60 ± 0.09	1.265 ± 0.003	0.70 ± 0.13	0.788 ± 0.002
	Folpet concentration (mg kg^{-1})			
	IR	LC		
Rinsate samples				
M1	1130 ± 190	1350 ± 118		
M2	4403 ± 145	4219 ± 128		
M3	9075 ± 290	11163 ± 173		
End of line	12530 ± 570	12137 ± 133		

4. Conclusions

An IR spectroscopy based methodology has been successfully developed and validated to implement contamination prevention programs in the pesticide industry. The method avoid traditional sensitivity problems of IR procedures by means of the use of a lab-made transmission cell with an open upper side with an internal volume of 35 μL and an optical pathlength of 0.5 mm, which provides detection limits of the order of parts per million. Moreover, the intrinsic characteristics of IR spectroscopy become the developed procedure a fast alternative to chromatographic procedures, reducing solvent consumption and minimizing waste generation.

The two evaluated sampling methods, based on swab and rinsate analysis, provided useful information to the manufacturers to implement their contamination prevention programs. From the swab tests, it can be concluded that the surface material and, specially, the physical state of this material, is the most important parameter to obtain good recoveries, probably because the oxidation degree affects the porosity and the rugosity of the material and it drastically affects the recovery of analytes from oxidized surfaces.

The results obtained in the analysis of real samples clearly confirms that a cleaning step between production of different commercial products is strictly necessary to prevent cross contamination and the presence of traces (up to low % range) of undesired compounds in the commercialized products. Moreover, the IR methodology plays two roles in this implementation; one qualitative, identifying the pesticides presents in the mixers of the production lines and possibly in the next products and the other quantitative, determining, accurately, the amount of those pesticides present in the mixers and in the rinsates. In this way, it is possible to reduce effectively cross contamination in the pesticide industry without increase substantially the costs associated to this reduction.

Finally, the IR methodology has demonstrated that it is useful in the prevention of contamination of pesticides production lines devoted to the manufacturing of folpet and cymoxanil and it can be extended to most of the commercially available pesticide solid formulations.

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