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Multiresidue determination of pesticides in industrial and fresh orange juice by hollow fiber microporous membrane liquid–liquid extraction and detection by liquid chromatography–electrospray-tandem mass spectrometry

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ABSTRACT

A procedure involving hollow fiber microporous membrane liquid–liquid extraction (HF-MMLLE) and detection by liquid chromatography with tandem mass spectrometry was developed and applied. The extraction is based on liquid–liquid microextraction with a polypropylene porous membrane as a solid support for the solvent. On the membrane walls the solvent forms a renewable liquid membrane which improves the trueness of the method and promotes the sample clean-up. The applicability of this method was evaluated through the simultaneous extraction of 18 pesticides of different classes: polar organophosphates, carbamates, neonicotinoids, amides, pyrimidines, benzimidazoles and triazoles in industrial and fresh orange juice. The parameters affecting the extraction efficiency were optimized by multivariable designs. Under optimized conditions, analytes were concentrated onto 1.5 cm long microporous membranes placed directly into the sample containing 9 mL of juice at pH 7.0, 4 g of ammonium sulfate and 400 μL of toluene:ethyl acetate (85:15, v/v). The best extraction conditions were achieved at 25 °C with 35 min of extraction time. The analyte desorption was carried out using 50 μL of methanol:acetone (50:50, v/v) for 2 min in an ultrasonic bath. Limits of detection ranging between 0.003–0.33 mg L^{-1}, 0.003–0.35 mg L^{-1} and 0.003–0.15 mg L^{-1} were obtained for the carton orange juice, carton light orange juice and fresh orange juice samples, respectively. Good repeatability (lower than 7.6%) was obtained for all three sample types. The method was applied to five different juice samples containing soybean extract, orange pulp, nectar, light juice and fresh orange juice. The results suggest that the proposed method represents a very simple and low-cost alternative microextraction procedure rendering adequate limits of quantification for the determination of these pesticides in juice samples.

1. Introduction

The use of chemical pesticides in fruit crops is necessary to control pests that could decrease the field production, as well as to improve the quality of the fruit which reaches the consumer. Also, pesticides can be applied on fruits for post-harvest protection and thus pesticide residues may be transferred from the fruit to the juice, this being a significant route to human exposure [1]. The risk of pesticide residues to human health is dependent on their ability to cause adverse health effects and the potential for exposure to the residues in the diet. There is a strict legislative framework controlling the use of such substances which aims to minimize the risks to human health associated with the consumption of residues. The Brazilian government has set tolerance levels for these compounds in the form of maximum residue limits (MRLs), which are in the range of parts-per-million (mg L^{-1}) [2,3].

Hence, nowadays there is an increasing demand for sensitive and selective methods for the determination of multi-class pesticides in fruit juices and fresh fruit at trace levels [1]. The difficulty in developing such multi-class pesticide determinations is compounded by the widely varying physicochemical properties of the different chemical classes of pesticide [4]. Among the analytical approaches used in residue control, liquid chromatography (LC) is effective in separating non-volatile and thermally labile compounds as well as pesticides compatible with gas chromatography (GC). LC–MS using either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) is a powerful tool for the trace determination of more complex pesticides, because the

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sensitivity is higher than that of HPLC performed with conventional detectors and the selectivity is improved by the selection of specific ionic fragments [4–9]. One disadvantage of these types of ionization, that is, the limited fragmentation compared with EI ionization used in GC–MS, can be addressed by employing tandem mass spectrometry (MS/MS). Both LC–MS and LC–MS/MS have been applied to the determination of a variety of pesticides belonging to different chemical classes [4–9]. In addition, an issue of utmost importance for the successful application of LC–MS/MS analysis is that of the unequivocal confirmation of the target compounds. Confirmation of positive samples is based on the principle of the maximum number of identification points (IPs) proposed by European Commission Decision 2002/657/EC and by "Quality Control Procedures for Pesticide Residues Analysis", SANCO No. 10684/2009 [4].

The analysis of complex matrixes like fresh fruits juice or commercial juices comprises a sample preparation step involving the clean-up and enrichment of analytes. Traditionally, liquid–liquid extraction (LLE) has been employed for this purpose. However, LLE is time consuming and also uses large volumes of toxic organic solvents. Other techniques considered traditional, like SPE and more recently the QuEChERS and ultrasonic solvent extraction, have been employed but, as described for LLE, these techniques are also laborious, time consuming and/or involve high solvent consumption [10–13]. Consequently, various extraction techniques have been developed in order to overcome these problems. Among these techniques, solid-phase microextraction (SPME) and, more recently, stir bar sorptive extraction (SBSE), and variants of the liquid-phase microextraction technique seem to be very promising [14–16].

A new configuration employing liquid-phase microextraction is hollow fiber microporous membrane liquid–liquid extraction (HF-MMLLE), where the liquid phase microextraction is supported by a microporous polypropylene membrane that works as a solid support and favors the formation of a renewable liquid membrane on the surface. This system presents more stability when compared with single drop microextraction (SDME) and better reproducibility and extraction efficiency. In this system liquid desorption by an organic solvent is used before the instrumental analysis. This feature of the system is convenient because it enables the technique to be employed for analysis through liquid and gas chromatography, and thus the properties of the analytes are the main limitation of the method [17].

In this study, a simple and low-cost methodology based on the simultaneous application of liquid–liquid microextraction (LLME) and microporous membrane solid-phase extraction (MMSPE), that is, HF-MMLLE, is presented. The proposed procedure was applied to the concentration, isolation and LC–MS/MS analysis for determination of the residues of 18 pesticides belonging to different chemical class (polar organophosphates, carbamates, neonicotinoids, amides, pyrimidines, benzimidazoles and triazoles) in orange juice and fresh juice samples. Multivariate optimization of several variables potentially affecting the microextraction procedure was performed. The trueness and precision of the method were evaluated through the recovery and within-laboratory reproducibility, respectively.

2. Experimental

2.1. Chemicals and reagents

Water obtained from a Milli-Q® UltrapureWater Purification System (Millipore, Brussels, Belgium), ethyl acetate, hexane, acetone, toluene, acetonitrile (Tedia, USA), ammonium sulfate, sodium chloride, sodium hydroxide (Synth, Brazil), methanol (JT Baker, Netherlands), formic acid, ammonium acetate (Merck, Germany) were used in this study. Diuron obtained from Chem Service (USA), and diflubenzuron, trichlorfon, pirimiphos-methyl, imidacloprid, abamectin, bromacil, ametryn, acetamiprid, acephate, diazinon, carbofuron, carbendazim, difenoconazole, malathion, tebuconazole, chlorpyrifos, thiophanate-methyl purchased from Pestanal (Germany) were the pesticide standards studied. The standard solutions were prepared in methanol and the working solutions were prepared in methanol:water (50:50) and stored at −18 °C. The industrial juice and fresh fruit samples were purchased at a local market.

A Q 3/2 Accurel polypropylene hollow fiber membrane (600 μm i.d., 200 μm wall thickness and 0.2 μm pore size) was purchased from Membrane GmbH (Wuppertal, Germany). The hollow fiber was cut into segments of 1.5 cm length, cleaned in acetone and dried before use.

2.2. Sample preparation

The extraction of pesticides in commercial orange juice and fresh juice was performed using the HF-MMLLE system. Into a 20 mL vial, 9 mL of juice, which was centrifuged for 5 min, 4 g of ammonium sulfate, adjusted to pH 7.0 with sodium hydroxide 0.1 mol L^{-1} and 400 μL of toluene:ethyl acetate (85:15, v/v) solution were added. A piece (1.5 cm length) of the fiber was washed with acetone, dried and fixed to a cylindrical stem. The stem was fixed through a septum of silicone to help seal the 20 mL vial contained 9 mL of sample and 400 μL of a mixture of toluene and ethyl acetate (85:15, v/v). The system was maintained under constant temperature and stirred during the entire extraction process. Under optimal conditions, the extraction time and temperature were 35 min and 25 °C, respectively. Subsequent to the extraction process, a desorption step was applied where the membrane containing the organic solvent enriched with the analytes was immersed in 50 μL of a methanol:acetone (50:50, v/v) mixture for 2 min in an ultrasonic bath. Desorption was carried out in a 100 μL microtube. After this process, 20 μL of the extract was injected into the LC system.

2.3. Instrumental analysis

Analysis was carried out on a Quattro Micro API quadrupole mass spectrometer coupled to an Alliance 2690 liquid chromatograph (Waters, Manchester, UK). The chromatograph was equipped with a Nova-Pak C18-A HPLC column (150 mm × 3.9 mm, 4 μm) manufactured by Waters, kept at 20–30 °C. The mobile phase consisted of acetonitrile and water acidified with 0.1% of formic acid and 5 mmol L^{-1} of ammonium acetate. A gradient was applied at a flow rate of 0.4 mL min^{-1} as follows: initial conditions of 25% acetonitrile in water phase held for 5 min, increased linearly to 35% in 5 min, increased linearly to 45% in 5 min, increased linearly to 60% in 5 min, increased linearly to 75% in 3 min, increased linearly to 85% in 2 min, increased linearly to 95% in 3 min, held at 95% for 3 min, returned to initial conditions in 0.1 min and maintained for 5 min. The quadrupole mass spectrometer was equipped with a Z-spray source for positive electrospray ionization (ESI). Capillary and cone voltages were set at 3.5 kV and 35 V, respectively. The temperature source was kept at 120 °C while desolvatation temperature was held at 450 °C. Nitrogen was used as the cone and desolvating gas at flow rates of 50 and 650 L h^{-1}, respectively. The mass spectrometer was operated in MS/MS mode using multiple reactions monitoring (MRM). Argon (99.8% pure) from Air Liquide (Rio de Janeiro, Brazil) was used as the collision gas at a constant pressure of 2×10^{-3} mbar. Table 1 summarizes the acquisition window definition, masses of parent and daughter ions that are

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Table 1
Selected ion transitions and instrumental parameters for the pesticides under study.

Pesticide	t_R (min)	SRM transitions (m/z)	Cone voltage (V)	Collision energy (eV)
Acephate	3.67	184.0 → 142.6[a]	17	7
		184.0 → 124.5[b]	17	18
Carbendazim	6.15	192.0 → 159.7	25	15
		192.0 → 131.7	25	30
Acetamiprid	9.08	223.1 → 125.5	28	17
		223.1 → 186.9	28	13
Imidacloprid	7.82	256.0 → 174.8	26	17
		256.0 → 209. 0	26	12
Trichlorfon	6.42	256.9 → 108.3	25	16
		256.9 → 126.5	25	15
Ametryn	19.93	228.1 → 185.8	34	18
		228.1 → 115.4	34	27
Bromacil	13.26	260.9 → 204.8	21	13
		260.9 → 187.7	21	27
Carbofuran	16.68	222.1 → 164.8	20	10
		222.1 → 122.5	20	19
Thiophanate-methyl	15.84	343.0 → 150.7	26	18
		343.0 → 310.9	26	10
Diuron	19.53	233.2 → 71.5	28	16
		233.2 → 159.8	28	25
Pirimiphos-methyl	29.43	306.1 → 107.4	40	32
		306.1 → 163.8	40	23
Tebuconazole	25.42	308.1 → 150.7	31	23
		308.1 → 124.5	31	36
Diflubenzuron	25.63	311.1 → 157.7	18	7
		311.1 → 140.6	18	31
Malathion	25.84	331.1 → 126.5	22	11
		331.1 → 210.8	22	19
Diazinon	28.53	305.1 → 168.8	33	20
		305.1 → 152.7	33	21
Difenoconazole	28.23	406.1 → 250.9	34	27
		406.1 → 336.9	34	16
Chlorpyrifos	31.15	349.9 → 197.8	23	16
		349.9 → 152.6	23	13
Abamectin [Na^+]	32.33	895.8 → 751.5	48	59
		895.8 → 182.9	48	41

The same sequence for all compounds.
[a] Quantifier.
[b] Qualifier ion

monitored, and the optimized collision induced dissociation (CID) voltages.

2.4. Optimization strategies

The optimization of the parameters affecting the extraction of the pesticides using the HF-MMLLE was performed using multivariate designs. A triangular surface mixture design was used to define the best extracting organic solvent for the extraction step (hexane, toluene and ethyl acetate) and desorption step (methanol, acetone and acetonitrile). A central composite design was applied to study the influence of sample pH, sample volume, extraction time, extraction temperature and solvent volume on the extraction efficiency. The results obtained for these studies were applied to the juice samples.

A univariate study to determine the influence of mass and type of salt on the extraction efficiency of the pesticides from juice and fresh fruit samples was evaluated. The best experimental conditions obtained were then applied to the analysis. In order to maximize the simultaneous extraction of the pesticides, the geometric average of the peak areas for the pesticides were used as the response, since good levels of detection for all pesticides and similar extraction conditions for coextracted compounds were obtained. The experimental data were processed using the Statsoft Statistica 6.0 computer program to evaluate the agreement of the model with the experimental design.

3. Results and discussion

3.1. Effect of volume and sample pH

The physicochemical features of pesticides influence the extraction efficiency in this system. The extraction occurs through interaction between the analytes and the organic solvent, thus a high partition coefficient between the sample and organic solvent is necessary. The target pesticides have different pKa values, which may have been ionized in acidic or very alkaline pH. Thus, there is the necessity to ensure the simultaneous extraction of the pesticides. The sample pH can also influence the release of pesticides from the matrix, hindering the interaction between the analytes and matrix, favoring the extraction efficiency. The sample volume was studied keeping the mass of the analyte constant to determine the effect of the matrix on the extraction. Fig. 1 shows the results obtained for the studied on the effect of the interaction between pH and sample volume on the extraction efficiency. The sample volume showed an effect on the extraction efficiency, since an increase in the sample volume promoted an increase in the analytical signal. Therefore, the sample volume selected was 9 mL. On the other hand, sample pH did not show a notable influence and a sample pH of 7 was selected for the other experiments.

3.2. Effect of salt

As mentioned above, the extraction efficiency is dependent on the partition coefficient of each pesticide between the sample and

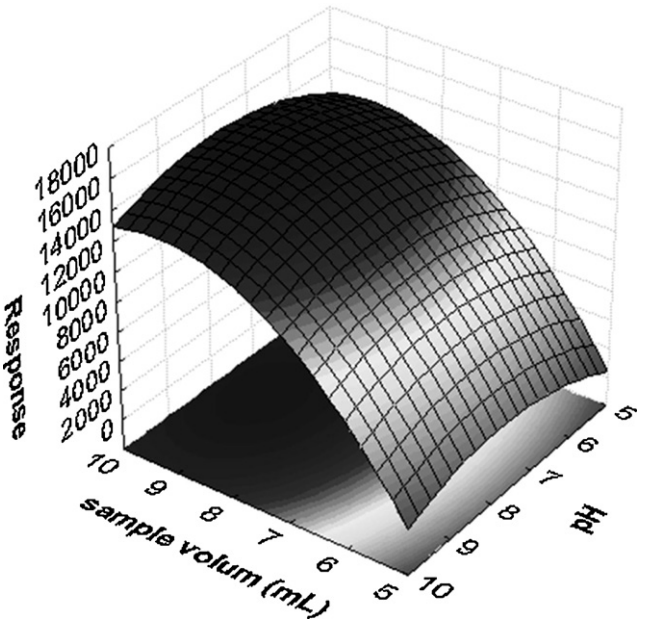

Fig. 1. Effect of sample volume and pH on the efficiency of pesticide extraction by HF-MMLLE. Experimental conditions: 4 g of sodium chloride, 500 μg L^{-1} of each pesticide, time extraction 30 min, room temperature, 150 μL of toluene as extraction solvent and 50 μL of methanol as desorption solvent.

organic solvent. This coefficient can be modified by adding an inert electrolyte, which is known as the salting-out effect. Thus, the addition of different salts and different masses was studied to obtain the optimal conditions for the extraction of the pesticides in the proposed method. In general, on increasing the ionic strength the extraction of analytes is improved. This behavior was obtained for both sodium chloride and ammonium sulfate, which presented similar extraction efficiency, as shown in Fig. 2. This behavior can be explained by the effect known as ionic suppression. Ion suppression is a matrix effect associated with liquid chromatography–mass spectrometry (LC–MS) techniques regardless of the sensitivity or selectivity of the mass analyzer used. Ion suppression negatively affects several analytical figures of merit, such as detection capability, precision, and trueness. The limited information available regarding the origin and mechanism of ion suppression makes

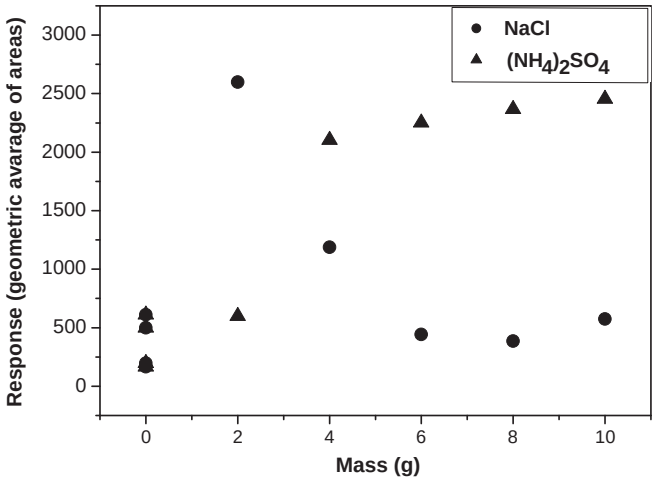

Fig. 2. Effect of addition of NaCl and $(NH_4)_2SO_4$ on the extraction efficiency. Experimental conditions: 500 μg L^{-1} of each pesticide, 9 mL of orange juice, pH 7, extraction time 30 min, room temperature, 150 μL of toluene as extraction solvent and 50 μL of methanol as desorption solvent.

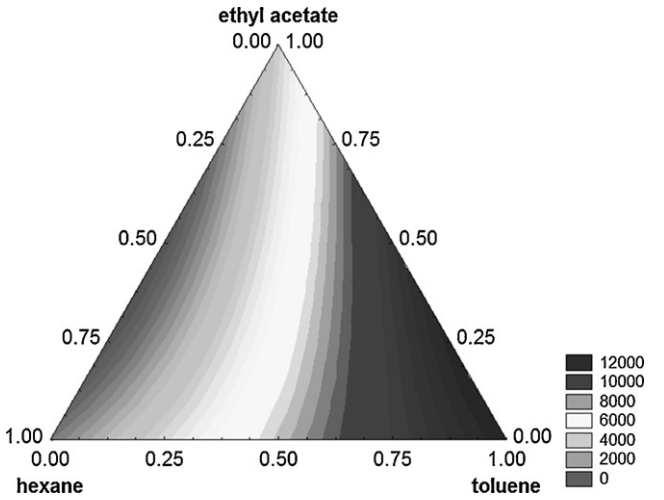

Fig. 3. Effect of different solvents used for the pesticide extraction (toluene, hexane and ethyl acetate) by HF-MMLLE on the pesticide response. Experimental conditions: 500 μg L^{-1} of each pesticide, 9 mL of orange juice, pH 7, 4 g of ammonium sulfate, extraction time 30 min, room temperature, 150 μL of solvent as extraction solvent and 50 μL of methanol as desorption solvent.

this problem difficult to solve in many cases. Modifying instrumental components and parameters, chromatographic separation, and sample preparation are all considered as means of reducing or possibly eliminating ion suppression [18]. There are many possible sources for ion suppression, including endogenous compounds from the sample matrices as well as exogenous substances, molecules not present in the original sample but from contamination during sample preparation, such as polymers extracted from different brands of plastic tubes or salts [19]. Although the mechanisms through which suppression occurs are not fully known, two possible causes of this effect can be proposed. In MRM monitoring the m/z ratio of the precursor ion is defined without sodium for the analytes that do not present an adduct. In the presence of excessive sodium in solution the formation of an adduct $[M–Na]^+$ that has a different m/z ratio occurs which leads to a notable loss in the method sensitivity. Also, the loss of sensitivity originating from the addition of NaCl may be related to the influence of the presence of

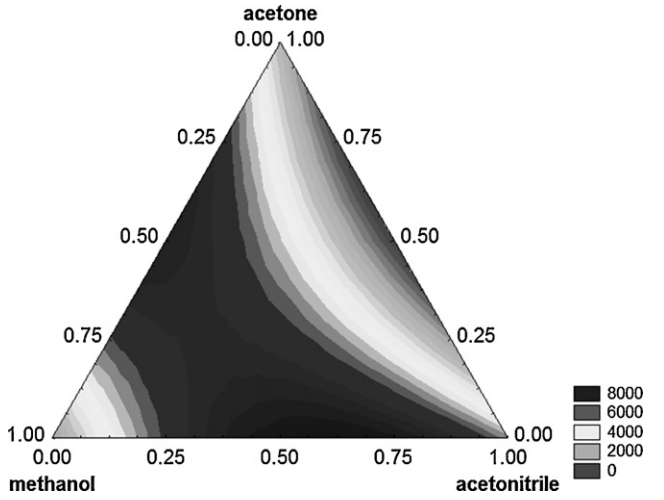

Fig. 4. Effect of different solvents used for liquid desorption (methanol, acetonitrile and acetone) after pesticide extraction by HF-MMLLE on the pesticide response. Experimental conditions: 500 μg L^{-1} of each pesticide, 9 mL of orange juice, pH 7, 4 g of ammonium sulfate, extraction time of the 30 min, room temperature, 150 μL of solvent as extraction solvent (toluene:ethyl acetate, 85:15, v/v) and 50 μL of solvent as desorption solvent.

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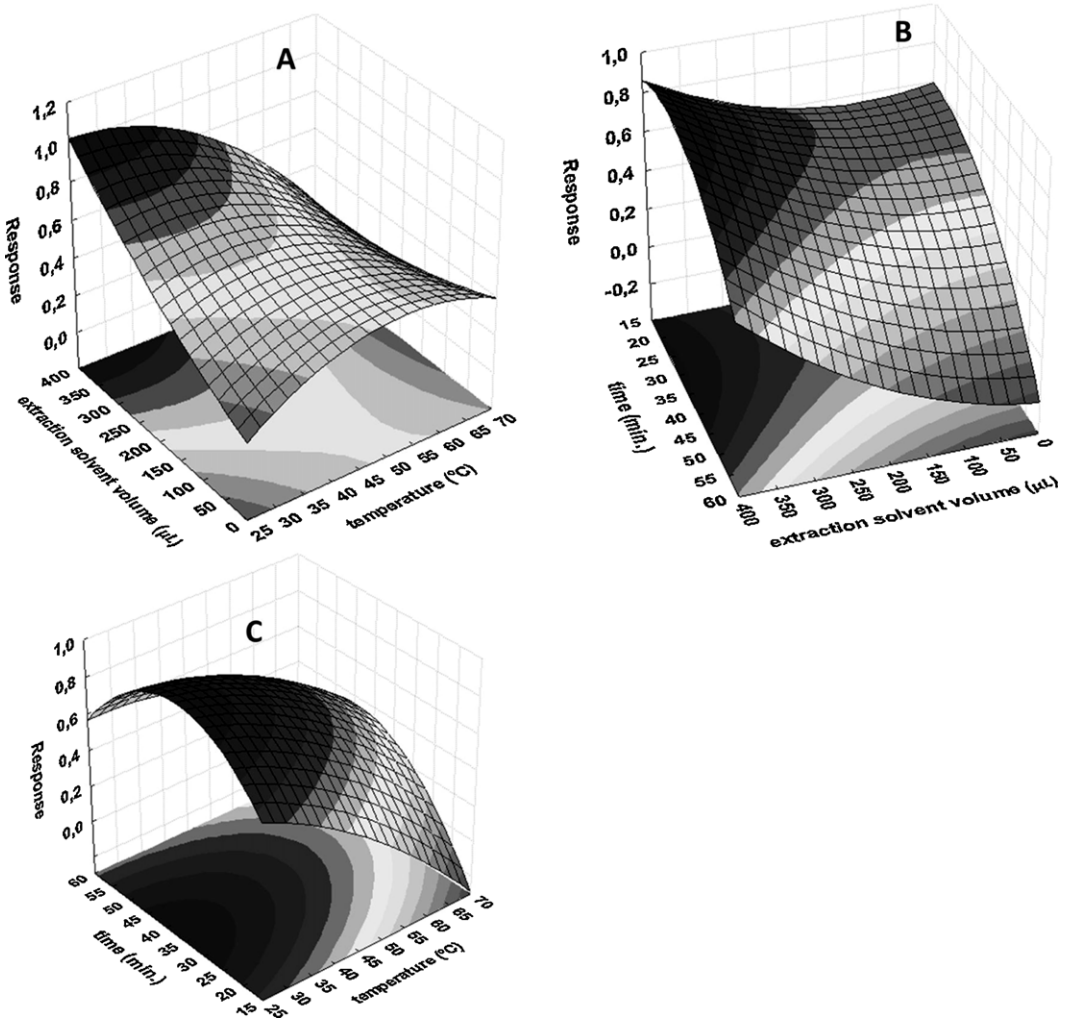

Fig. 5. Surface response to optimize the variables extraction solvent volume, extraction time and extraction temperature. (A) Effect of extraction temperature and extraction solvent volume on the extraction efficiency. (B) Effect of extraction time and extraction solvent volume on the extraction efficiency. (C) Effect of extraction temperature and time on the extraction efficiency. Experimental conditions: sample volume 9 mL, desorption solvent volume 50 μL (methanol:acetone, 50:50, v/v), extraction temperature from 25 to 70 °C, extraction time from 15 to 60 min and extraction solvent volume between 0 and 400 μL.

sodium when using the ionization source in question, that is, electrospray (ESI). In this source, charged microdroplets containing the analyte and the mobile phase are formed. After the solvent evaporation, a decreasing in the droplet size occurs and the ions are ejected and move to the inside of the mass spectrometer to be analyzed. At this stage the presence of sodium becomes critical and due to the level of solvation it tends to stay inside the droplet and adducts are formed. Furthermore, the presence of sodium in excess can hinder the formation of droplets in the electrospray process. There is the possibility that the analytes and NH_4^+ ions $[M–NH_4]^+$ interact and form adducts. Therefore, for the pesticides in this study, as seen in Fig. 2, no pronounced effect is observed. Fig. 2 shows that the best analytical signal is obtained when 2 g for sodium chloride and 4 g of ammonium sulfate are used. Since the response using the aforementioned mass of sodium chloride was significantly higher compared to that using ammonium sulfate, a analysis of the relative standard deviation was carried out (RSD, $n = 6$) for extractions using 2 g of sodium chloride and 4 g of ammonium sulfate because, as previously mentioned, the ion suppression may compromise the precision and trueness of the method. The RSDs found using sodium chloride were above 57% while using ammonium sulfate they remained at 15%. Thus, the mass of salt selected to continue the experiments was the 4 g of ammonium sulfate.

3.3. Selection of solvent extraction and solvent desorption

Since the proposed methodology is based on the partitioning between the analyte and the organic extraction solvent, this solvent should present the highest possible capacity of interaction with the analytes. At the same time, the extraction solvent should have the potential to extract all of the analytes simultaneously, maintaining features such as a high affinity with the membrane and low volatility, toxicity and water solubility. The methodology involves the extraction of pesticides from different classes with different polarities and interactions with the sample; therefore it is possible for a mixture of solvents with different polarities to exert greater influence on the extraction efficiency. The solvent extraction study was performed using the solvents toluene, hexane and ethyl acetate on a triangular surface which considers the binary and ternary mixture of solvents as well as each one separately. Fig. 3 shows that a mixture of toluene and ethyl acetate (85:15, v/v) has higher efficiency and thus this was selected as the extraction solvent for the other experiments.

After the extraction procedure, the liquid desorption step, in which the membrane remains immersed in 50 μL of desorption solvent, was performed. The analytes must be highly soluble in the desorption solvent and also present compatibility with the mobile

phase and with the instrumental detection technique employed. As in the case of the extraction solvent, the desorption solvent was studied using the triangular surface methodology. Fig. 4 shows that there is equivalence in extraction efficiency when employing a solution of methanol and acetonitrile (50:50, v/v) or methanol and acetone (50:50, v/v). A solution of methanol and acetone (50:50, v/v) was selected for the other experiments.

3.4. Effect of extraction solvent volume, time and temperature

The variables extraction solvent volume (0; 100; 200; 300 and 400 μL of toluene:ethyl acetate, 85:15, v/v), extraction time (15; 26.3; 37.5; 48 and 60 min) and extraction temperature (25; 36.25; 47.5; 58.75 and 70 °C) were optimized using a central composite design.

From the results obtained, the combinations of the three factors were plotted generating three response surfaces (Fig. 5). Thus, quadratic regression equations were obtained for each response surface and the optimum value for each factor was obtained. For extraction techniques based on the diffusion of the analytes, the extraction time and temperature would be expected to have an important effect on the extraction efficiency. The optimum time and temperature of the extraction are dependent on the sample and analytes. Fig. 5A shows that the temperature does not have a significant effect on the extraction efficiency allowing the extraction to be carried out at room temperature. The solvent volume tends toward a maximum which may be due to the complex composition of the sample. Any loss of solvent may be due to the formation of a microemulsion. It was also possible to observe that in the absence of solvent the extraction occurs only through the membrane, however, with considerably lower efficiency when compared to the extraction in the presence of the solvent. The tendency toward the maximum volume of solvent is confirmed when the extraction time and solvent volume effects were studied (Fig. 5B). It was shown that the volume of the solvent tends toward the maximum while the time remains closed to the central point, enabling high analytical frequency. The effect observed for the extraction time may be due to the formation of a microemulsion in the sample, considering that the solvent volume is 400 μL, thereby causing a loss of analytical signal for extended periods. Fig. 5C is in agreement with previous surfaces presenting little influence of the temperature and an optimum extraction time near the central point. Besides the complexity of the matrix the diversity of the analytes must be considered in order to understand the effects produced. In relation to molecular mass variation (an important factor given that the process is controlled by diffusion) there are analytes with masses varying from 183.17 g mol^{-1} (acephate) to 866.60 g mol^{-1} (abamectin), and differences in terms of the polarity and interaction between matrix components. The most important issue to be considered is obtaining good conditions for the efficient extraction of all pesticides simultaneously. Thus, the region of maximum response corresponds to a temperature of 25 °C, extraction time of 35 min and extraction solvent volume of 400 μL (toluene:ethyl acetate, 85:15, v/v). These values were then applied for the remainder of this study.

3.5. Analytical figures of merit and trueness

From the results obtained in the optimization procedure, the analytical figures of merit were investigated for each type of sample (regular carton orange juice, light carton orange juice, fresh orange juice). Calibration curves were constructed to estimate the linear range, correlation coefficients, and limits of detection and quantification for the proposed HF-MMLLE method. The limits of detection and quantification were calculated as three and ten times the signal to noise ratio, respectively. The validation was performed

Table 2
Linear range, relative recoveries, repeatability and precision, correlation coefficients, and limits of detection and quantification obtained for the proposed method to determine pesticides in carton orange juice samples using the polypropylene membrane for extraction and LC/MS/MS for determination.

Compound	Linear range (mg L^{-1})	r	LOD[a] (mg L^{-1})	LOQ[b] (mg L^{-1})	Relative recovery %(mg L^{-1})					Repeatability (0.5 mg L^{-1}/n = 6)%	Intermediateprecision (0.5 mg L^{-1}/n = 6)%
					0.1	0.3	0.5	2.5	9.0		
Acephate	0.3–10	0.999	0.15	0.50	–	66–95	85–107	92–100	83–80	4.7	7.4
Carbendazim	0.3–7	0.999	0.11	0.37	–	76–95	93–101	100–116	–	6.9	11.0
Acetamiprid	0.05–3	0.999	0.018	0.060	109–116	70–97	101–107	89–115	–	6.0	11.4
Imidaclorprid	0.5–10	0.991	0.16	0.54	–	–	118–118	85–88	–	5.9	13.8
Trichlorfon	0.05–7	0.995	0.02	0.06	107–116	91–110	90–111	77–91	–	5.6	12.9
Ametryn	0.01–1	0.997	0.003	0.010	87–112	73–117	94–100	–	–	6.9	14.6
Bromacil	0.05–3	0.999	0.022	0.070	81–87	104–120	90–96	110–113	–	7.3	12.7
Carbofuran	0.03–0.5	0.999	0.011	0.036	82–104	105–121	95–101	–	–	6.9	11.9
Thiophanate-methyl	0.3–10	0.998	0.07	0.25	–	85–120	116–118	94–101	90–118	7.4	12.7
Diuron	0.05–5	0.999	0.03	0.09	108–110	85–114	103–107	116–121	87–116	7.1	11.3
Pirimiphos-methyl	0.5–10	0.991	0.30	0.99	–	–	100–104	93–116	114–115	7.1	15.4
Tebuconazole	1.0–10	0.996	0.33	1.11	–	–	–	84–120	87–116	5.8	12.6
Diflubenzuron	0.1–5	0.997	0.04	0.13	89–107	92–109	110–119	98–103	–	6.8	14.8
Malathion	0.5–5	0.991	0.26	0.85	–	–	102–116	104–117	–	6.8	14.3
Diazinon	0.3–10	0.997	0.10	0.33	–	72–78	96–101	76–79	90–120	7.4	15.5
Difenoconazole	0.2–10	0.998	0.06	0.20	–	86–101	104–104	88–121	78–116	6.5	13.6
Chlorpyrifos	0.2–10	0.998	0.07	0.23	–	99–102	85–119	87–121	96–116	7.6	15.5
Abamectin	0.2–10	0.992	0.09	0.27	–	79–99	86–96	93–100	95–120	7.0	14.2

[a] LOD – Limit of detection.
[b] LOQ – Limit of quantification.

Table 3
Linear range, relative recoveries, correlation coefficients, and limits of detection and quantification obtained for the proposed method to determine pesticides in carton orange juice light samples using the polypropylene membrane for extraction and LC/MS/MS for determination.

Compound	Linear range ($mg\,L^{-1}$)	R	LOD ($mg\,L^{-1}$)	LOQ ($mg\,L^{-1}$)	Relative recovery % (500 $mg\,L^{-1}$)				
					0.1	0.3	0.5	2.5	9.0
Acephate	0.3–5	0.988	0.13	0.42	–	109–118	98–101	104–109	–
Carbendazim	1–10	0.994	0.35	1.16	–	–	–	86–120	117–120
Acetamiprid	0.1–3	0.999	0.04	0.13	96–103	84–117	100–121	99–108	–
Imidaclorprid	0.3–10	0.999	0.11	0.36	–	62–69	71–94	78–117	83–99
Trichlorfon	0.05–7	0.995	0.02	0.06	107–116	91–110	90–111	77–91	–
Ametryn	0.01–1	0.993	0.003	0.010	90–119	93–99	85–93	–	–
Bromacil	0.05–3	0.998	0.02	0.06	80–93	66–73	86–90	92–99	–
Carbofuran	0.03–0.5	0.999	0.01	0.03	73–92	82–102	74–91	–	–
Thiophanate-methyl	0.5–10	0.998	0.17	0.56	–	–	116–120	101–106	75–104
Diuron	0.02–1	0.999	0.008	0.02	79–86	117–121	98–104	–	–
Pirimiphos-methyl	0.5–10	0.998	0.20	0.68	–	–	109–115	97–99	83–87
Tebuconazole	1–10	0.992	0.49	1.60	–	–	–	104–107	92–96
Diflubenzuron	0.05–5	0.999	0.03	0.09	97.41–101.62	101–101	68–100	73–77	–
Malathion	0.5–10	0.987	0.21	0.71	–	–	112–115	84–106	99–111
Diazinon	0.3–5	0.994	0.11	0.36	–	81.56–87.69	102–119	106–111	–
Difenoconazole	0.3–5	0.994	0.12	0.39	–	87–116	109–111	81–82	–
Chlorpyrifos	0.2–10	0.998	0.07	0.23	–	99–102	85–119	87–121	96–116
Tebuconazole	1.0–10	0.996	0.33	1.11	–	–	–	84–120	87–116

with regular and light juice (from the carton) and fresh natural juice due to their differences in composition. The light juice contains other constituents in its composition in addition to those of the regular juice including xanthan gum, sucralose and acesulfame potassium. These components influence the pesticide extraction efficiency and may cause ion suppression. The results obtained for the regular juice, light juice and fresh orange juice samples are summarized in Tables 2–4, respectively. Good correlation coefficients (r) were obtained for all matrices studied. For the carton orange juice sample, the method showed an excellent repeatability and intermediate precision, calculated as the relative standard deviation ($n=6$) using solutions spiked with 500 $\mu g\,L^{-1}$ of each pesticide, in the range of 4.7–7.4% and 7.4–15.4%, respectively. Relative recovery assays were carried out for the regular juice, light juice and fresh orange juice using different levels of concentration (100 $\mu g\,L^{-1}$, 300 $\mu g\,L^{-1}$, 500 $\mu g\,L^{-1}$, 2500 $\mu g\,L^{-1}$ and 9000 $\mu g\,L^{-1}$ for all samples) showing excellent results considering the complexity of the samples. The pesticides trichlorfon, abamectin and chlorpyrifos cannot be quantified due to ion suppression occurring following interaction with some components of the light juice matrix. The proposed method presented good LOD and LOQ values, probably because of the excellent sample clean-up promoted by the membrane, verifying its suitability for the determination of pesticides in these types of orange juice. The LOD values for the proposed procedure are similar to those obtained for methods based on HF-LPME (hollow fiber liquid phase microextraction) applied to similar samples.

3.6. Application of the methodology to orange juice samples

The proposed method was also applied to the analysis of different orange juice samples purchased at a supermarket in the city of Campinas in São Paulo, Brazil. The analytes were quantified using the addition calibration technique and recovery tests were performed spiking each sample with 500 $\mu g\,L^{-1}$ of each pesticide for the light juice (from the carton) and juice (from the carton) with soy extract, and the other types of orange juice showed no significant differences in relation to these calibration curves. This procedure

Table 4
Linear range, relative recoveries, correlation coefficients, and limits of detection and quantification obtained for the proposed method to determine pesticides in fresh orange juice samples using the polypropylene membrane for extraction and LC/MS/MS for determination.

Compound	Linear range ($mg\,L^{-1}$)	R	LOD ($mg\,L^{-1}$)	LOQ ($mg\,L^{-1}$)	Relative recovery % ($mg\,L^{-1}$)				
					0.1	0.3	0.5	2.5	9.0
Acephate	0.3–3	0.992	0.12	0.39	–	75–85	98–117	72–90	–
Carbendazim	0.3–7	0.992	0.13	0.46	–	78–113	73–119	84–103	–
Acetamiprid	0.3–7	0.995	0.10	0.33	–	68–96	73–86	78–96	–
Imidaclorprid	0.3–7	0.992	0.13	0.46	–	64–107	71–112	77–83	–
Trichlorfon	0.05–7	0.998	0.03	0.09	114–121	100–120	106–118	62–76	–
Ametryn	0.01–1	0.991	0.003	0.010	74–98	115–120	81–84	–	–
Bromacil	0.03–7	0.995	0.010	0.033	95–103	73–82	108–120	77–104	–
Carbofuran	0.03–10	0.996	0.01	0.04	99–114	97–113	107–118	73–98	102–103
Thiophanate-methyl	0.5–10	0.993	0.16	0.53	–	–	85–90	81–101	69–84
Diuron	0.05–10	0.999	0.029	0.096	75–79	111–120	91–113	83–98	107–113
Pirimiphos-methyl	0.2–10	0.992	0.07	0.25	–	102–112	87–90	106–116	96–111
Tebuconazole	0.5–7	0.992	0.15	0.50	–	–	96–104	105–118	–
Diflubenzuron	0.1–7	0.992	0.04	0.15	106–108	85–99	88–92	88–111	–
Malathion	0.5–7	0.996	0.15	0.49	–	–	95–97	73–97	–
Diazinon	0.5–7	0.995	0.14	0.46	–	–	91–100	76–94	–
Difenoconazole	0.1–7	0.991	0.06	0.19	97–99	102–103	94–102	91–102	–
Chlorpyrifos	0.2–10	0.994	0.08	0.26	–	79–108	104–107	111–118	82–107
Abamectin	0.2–10	0.995	0.06	0.21	–	104–119	91–112	90–110	86–96

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Table 5
Results obtained for the determination of the target pesticides determination in different orange juice samples using the HF-MMLLE–LC/MS/MS method and relative recoveries.

Compound	Carton orange juice with soybean extract		Carton orange juice (nectar)		Carton orange juice light		Carton orange juice with orange pulp ($mg\,L^{-1}$)	Fresh orange juice ($mg\,L^{-1}$)	MRL*
	Found ($mg\,L^{-1}$)	Relative recovery % (500 $\mu g\,L^{-1}$)	Found ($mg\,L^{-1}$)	Relative recovery % (500 $\mu g\,L^{-1}$)	Found ($mg\,L^{-1}$)	Relative recovery % (500 $\mu g\,L^{-1}$)			
Acephate	0.46 ± 0.05	63–105	0.90 ± 0.06	108–118	–	75–121	–	0.69 ± 0.09	0.5
Carbendazim	–	76–80	–	78–89	2.96 ± 0.21	107–120	–	–	5.0
Acetamiprid	–	77–111	1.53 ± 0.39	77–109	0.27 ± 0.01	65–109	–	–	0.5
Imidaclorprid	–	61–76	1.14 ± 0.17	92–120	0.55 ± 0.18	63–105	–	–	1.0
Trichlorfon	–	69–91	–	107–119	–	–	–	–	0.1
Ametryn	–	67–72	0.034 ± 0.001	73–114	0.48 ± 0.16	92–120	0.033 ± 0.002	–	0.02
Bromacil	–	67–76	0.20 ± 0.01	118–120	–	100–120	–	–	0.1
Carbofuran	–	69–77	1.22 ± 0.09	108–120	–	91–95	–	–	0.05
Thiophanate-methyl	–	108–113	0.88 ± 0.08	100–114	4.46 ± 0.82	99–120	–	–	5.0
Diuron	–	71–95	1.03 ± 0.05	100–118	–	101–105	–	–	0.1
Pirimiphos-methyl	–	80–93	–	75–100	1.13 ± 0.04	118–120	–	–	5.0
Tebuconazole	–	75–75	–	105–119	2.75 ± 0.10	80–121	–	–	5.0
Diflubenzuron	–	63–78	–	70–84	–	71–82	–	–	0.2
Malathion	–	102–107	1.55 ± 0.28	69–102	4.34 ± 0.09	84–109	–	–	4.0
Diazinon	–	66–69	0.77 ± 0.04	86–88	1.13 ± 0.02	97–107	–	–	0.7
Difenoconazole	–	61–90	0.83 ± 0.03	71–71	0.73 ± 0.03	81–91	–	–	0.5
Chlorpyrifos	–	63–68	0.43 ± 0.02	88–109	–	–	–	–	2.0
Abamectin	0.51 ± 0.10	99–117	–	95–112	–	–	–	–	0.005

* MRL – Maximum Residual Limits determined by ANVISA, Brazilian regulator agency. Unit, $mg\,kg^{-1}$.

was carried out in triplicate and the results can be observed in Table 5. It was not possible to obtain the maximum residual content allowed by ANVISA, which may be due to the high molecular mass which reduces the diffusion of the membrane. The pesticides acephate, acetamiprid, imidaclorprid, ametryne, bromacil, carbofuran, diazinon and difeconazole were found in concentrations above the maximum allowed. It was observed that the occurrence of pesticides in industrialized juices is significantly higher than in fresh natural juices, possibly due to the pre-concentration of the pulp during the production process, which is subsequently added to the juices.

4. Conclusions

The HF-MMLLE procedures to determine multiresidues of pesticides in different types of orange juice provided acceptable limits of detection ($mg\,L^{-1}$) and good precision and linearity. The proposed method presents some advantages and drawbacks of HF-LPME as it is simple, effective, and of low cost, uses microliters of organic solvents, is almost free of matrix effects, and completely avoids problems associated with carry-over. On the other hand, HF-LPME is relatively inefficient for the more polar substances. To the best of our knowledge, this constitutes the first study that applies HF-MMLE to the extraction of multiresidues of pesticides from industrial and fresh orange juice samples with acceptable clean up and trueness.

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