RESIDUES AND TRACE ELEMENTS

Use of Magnesium Silicate as a New Type of Adsorbent for Dispersive Solid-Phase Extraction Cleanup of the Quick, Cheap, Effective, Rugged, and Safe Method for Pesticides During Analysis of Lager Beer by Gas Chromatography-Tandem Mass Spectrometry

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Background: **Pesticides are applied for pest control during the production of cereal grains used in beer production. Given the risks for consumers, it is important to analyze the pesticide residues.** ***Objective:*** **Quick, easy, cheap, effective, rugged, and safe (QuEChERS)-based methods are very effective, and improvement in the cleanup step is an important approach.** ***Methods:*** **Primary secondary amine (PSA) and magnesium silicate were evaluated for dispersive-solid-phase extraction (d-SPE) cleanup step in extracts provided by the QuEChERS method in combination with GC-tandem MS for the determination of acetamiprid, terburfos, alachlor, ametryn, atrazine, azoxystrobin, carbofuran, carbosulfan, cypermethrin, deltamethrin, difenoconazole, esfenvalerate, flutriafol, thiamethoxam, and parathion-methyl in lager beer.** ***Results:*** **The amount of 50 mg of magnesium silicate was suitable for cleaning up beer extract as an alternative d-SPE material to PSA. The method was validated using beer fortified with pesticides at three concentration levels (0.002, 0.01, and 0.1 µg/mL). Average recoveries ranged from 70 to 123%, with RSDs between 0.3 and 10.5 %. Matrix effects were observed by comparing the slope of matrix-matched standard calibration with that of solvent. The method provided good linearity at the concentration levels of 0.001–2.5 µg/mL. Detection limits ranged from 0.0001 to 0.0007 µg/mL and quantification limits ranged from 0.001 to 0.006 µg/mL. The method was applied to nine beer brands.** ***Conclusions:*** **Results showed that magnesium silicate is an efficient alternative cleanup material to reduce analysis costs while maintaining the method reliability and accuracy.** ***Highlights:*** **Magnesium silicate was effective as adsorbent for d-SPE step in the analysis of pesticides in beer.**

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The contamination of food and beverages by pesticides is currently of major global concern becaus many of these compounds are detrimental to both human health and the environment (1). Beer is an alcoholic beverage made by the fermentation of grain. In the vast majority of the world's beers, the grain base is barley. However, many brewers use other grains along with barley to create their beer. Most of the beer sold in the world is made with rice or corn included in the grain variety. The alternate grains, like rice and corn, make the beer lighter than barley does, and that seems to be the goal for most makers of pale lager. The grains are treated with pesticides to protect crops from pests and control insect-borne diseases. Consequences of this application of pesticide include accumulation of the compounds in the food and beverages with potential risks for consumers (2, 3).

Determination of pesticides in lager beer samples relies on the use of a complex chromatographic instrumentation and the application of sample extraction procedures in order to isolate analytes, remove interfering substances, and achieve the sensitivity required for beverage control. This has been carried out using liquid–liquid extraction (LLE) with organic solvents (4), ultrasound-assisted ionic-liquid based dispersive liquid–liquid microextraction (5), as well as solid-phase extraction (SPE) with different adsorbents. The most widely used adsorbents are aminopropyl and polymeric resins, among others (6–8). More recently, the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method has been investigated using commercial adsorbent materials including primary and secondary amine and C_{18}-bonded silica (9–11). This method required shaking the sample with acetonitrile followed by shaking with sodium chloride and $MgSO_4$ to remove water. The salts create an exothermic reaction with water, induce phase separation between water and acetonitrile, and extract cleanup step by dispersive-SPE (d-SPE). Dispersive-SPE allows for the introduction of different amounts and types of sorbents so that the procedure can investigate pesticides that belong to different chemical classes (12). To the best of our knowledge, no multiresidue method based on QuEChERS using magnesium silicate as a cleanup adsorbent, followed by GC-MS/MS analysis, has yet to be reported in the literature. Therefore, our contribution is important in addressing this issue.

Considering lager beer is made with up to 30% rice or corn in addition to hops and barley malt, the present work describes a simple method for the simultaneous determination of

acetamiprid, alachlor, ametryn, atrazine, terbufos, azoxystrobin, carbofuran, carbosulfan, cypermethrin, deltamethrin, difenoconazole, esfenvalerate, flutriafol, thiamethoxam, and parathion-methyl residues in lager beer by QuEChERS, using magnesium silicate as an alternative adsorbent in d-SPE step, with detection by GC/MS. These pesticides were selected because they are commonly used for pest control in rice and corn. The proposed method was applied for the determination of pesticides in different lager beer brands.

Materials and Methods

Chemicals and Solvents

Pesticide certified standards (purity >97%) were obtained from the Dr. Ehrenstorfer (Augsburg, Germany). The GC grade solvent acetonitrile and acetic acid were purchased from Tedia (Fairfield, OH). Research grade magnesium silicate (60–100 mesh) and primary and secondary amines (50 µm) were supplied by Sigma (Büchs, Switzerland), C_{18}-bonded silica (50 µm) was obtained from Phenomenex (Torrance, CA), and anhydrous magnesium sulfate and sodium acetate were purchased from Macherey-Nagel (Düren, Germany).

Pesticide Standard Solutions

Individual stock standard solutions of the pesticides (1000 µg/mL) were prepared by weighing out 0.0250 g pesticide using an analytical balance with precision of 0.1 mg and a maximum capacity of 120 g (Model BL 2105; Sartorius, Göttingen, Germany), and dissolving it in 25 mL acetonitrile. The solutions were stored in a freezer at –18°C in glass bottles with PTFE-lined screw-caps. Calibration standards at concentrations of 0.001, 0.005, 0.01, 0.10, 0.25, 0.5, 1.0, 1.5, and 2.5 µg/mL were prepared by diluting the working standard solutions directly in the matrix extract obtained following the QuEChERS procedure.

GC-MS/MS Analysis

The separation of the pesticide residues from the extracts was carried out using a gas Model 2010 Plus gas chromatography system (Shimadzu, Kyoto, Japan) coupled to a Shimadzu TQ8040 triple quadrupole mass spectrometer, operated in multiple reaction monitoring (MRM) mode. Injections (1 µL volume) were performed using an AOC-5000 Plus (Shimadzu Instruments) autosampler in combination with a Model SPL-2010 Plus split/splitless injector containing a glass liner packed with deactivated glass wool. The chromatographic separation was performed using an SBL-5MS fused silica capillary column (30 m length × 0.25 mm i.d., 0.25 µm film thickness), supplied by Supelco (Bellefonte, PA), with helium (99.999% purity) as the carrier gas at a flow rate of 1.2 mL/min. Data acquisition and processing were performed with GCMS Solution v. 4.20 Workstation software (Shimadzu Instruments). The GC conditions were as follows: injector temperature of 250°C; initial oven temperature of 100°C (held for 1 min), followed by a ramp at 15°C/min to 300°C (held for 25 min). MS detector interface temperature of 280°C; source temperature of 250°C; splitless injection mode. The total GC run time was 31 min. Data acquisition was first performed in full scanning mode, from 50 to 500 *m/z*, in order to confirm the retention times of the analytes. All the standards and sample extracts were analyzed in MRM mode. The filament was switched off during a delay time of 5 min and was then switched on before elution of the first pesticide.

Sample Preparation and Fortification

Lager beer samples were collected from a commercial market located in the municipality of Aracaju, Brazil. Prior to the QuEChERS procedure, 300 mL lager beer was degassed in an ultrasonic water bath for 15 min to eliminate carbon dioxide content. Quantification was performed by external calibration, preparing the calibration standards in matrix-matched solutions. Fortified samples were prepared by adding 100 µL different standard solutions to 10 mL sample, resulting in concentration levels of 0.002, 0.01, and 0.1 µg/mL.

Extraction Procedure

A 10 mL portion of lager beer was placed into a polypropylene tube (approximately 50 mL capacity), followed by addition of 10 mL acetonitrile, containing 1% (v/v) acetic acid. The sample was vigorously shaken for 1 min. After this, 6 g anhydrous magnesium sulfate and 1.5 g sodium acetate were added, and the mixture was again shaken for 1 min, followed by centrifugation at 3500 rpm for 5 min. A portion of 1 mL organic phase was transferred in 15 mL polypropylene tube containing 150 mg anhydrous magnesium sulfate, 50 mg C_{18}, and 50 mg magnesium silicate. The mixture was again shaken for 1 min, followed by centrifugation at 3500 rpm for 5 min. Finally, a 1 µL portion of the extract was then directly analyzed by GC-MS/MS.

Results and Discussion

GC-MS/MS Conditions

In GC-MS/MS MRM mode, the MRM transition and collision energy (CE) must be optimized. MS/MS detection was performed by isolation of the selected parent ion for each compound, followed by application of adequate CE for its subsequent fragmentation. Precursor ions were selected from the EI spectra based on aspects including high *m/z* values, peak abundance, and the chromatographic signals obtained after isolating the ions in the analyzer. The product ions obtained were scanned over a characteristic mass range, resulting in the MS/MS spectrum. The retention times of the pesticides were determined using individual standard solutions at a concentration of 1.0 µg/mL. The GC-MS/MS instrument was operated in full scan mode, varying the oven temperature and the carrier gas flow rate. The most representative ions (the most intense ions) were selected for quantification of the pesticides in the lager beer samples (Table 1). During the analyses, it was found that the signal intensities changed as a result of the matrix components. This effect was assessed by comparing the instrumental responses (chromatographic peak areas) obtained for the pesticide solutions with those for solutions prepared in the sample extract (control) at the same concentrations.

For all the pesticides, the area values were found to be higher for the sample extracts. Identification of the compounds was achieved using the retention times obtained for injections

Table 1. Mass spectrometer parameters and retention times for pesticides in the positive ESI mode

Pesticide	Retention time, min	Quantification transition, *m/z*	CE, eV	Confirmation transition, *m/z*	CE, eV	Confirmation transition, *m/z*	CE, eV
Carbofuran	9.13	164.00 > 149.10	9	164.00 > 103.10	27	164.00 > 131.10	18
Atrazine	9.23	200.00 > 122.10	9	200.00>132.10	9	200.00 > 71.10	18
Terbufos	9.41	57.00 > 55.10	15	57.00 > 54.00	42	57.00 > 52.00	45
Alachlor	10.29	188.00 >160.10	9	188.00 > 131.10	24	188.00 > 146.10	15
Parathion-methyl	10.30	109.00 > 79.00	9	109.00 > 81.10	9	109.00 > 93.10	9
Ametryn	10.38	227.00 > 185.10	6	227.00 > 58.10	15	227.00 > 170.10	12
Thiamethoxam	11.26	212.00 > 139.10	12	212.00 > 182.10	6	212.00 > 125.10	9
Flutriafol	11.97	123.00 > 95.10	15	123.00 > 75.10	24	123.00 > 69.10	30
Carbosulfan	13.62	160.00 > 104.10	9	160.00 > 57.10	15	160.00 > 62.00	18
Acetamiprid	13.84	152.00 > 116.10	18	152.00 > 89.10	27	152.00 > 125.10	15
Cypemethrin 1	16.55	181.00 > 152.20	24	181.00 > 127.10	24	152.00 > 77.20	27
Cypermethrin 2	16.69	163.00 > 127.10	6	163.00 > 91.10	15	152.00 > 109.10	18
Cypermethrin 3	16.79	181.00 > 152.20	24	181.00 > 127.10	27	181.00 > 151.10	24
Cypermethrin 4	16.85	163.00 > 127.10	6	163.00 > 91.20	18	163.00 > 108.90	18
Esfenvalerate 1	18.09	125.00 > 89.10	18	125.00 > 99.10	21	125.00 > 63.00	27
Esfenvalerate 2	18.49	125.00 > 89.10	18	125.00 > 99.10	21	125.00 > 63.00	27
Difenoconaozle 1	19.11	265.00 > 202.00	21	265.00 > 139.10	27	265.00 > 209.00	18
Difenoconazole 2	19.25	265.00 > 202.00	18	265.00 > 139.100	27	265.00 > 209.00	15
Deltamethrin	19.71	181.00 > 152.10	24	181.00 > 127.10	27	181.00 > 77.00	30
Azoxistrobin	20.19	344.00 > 329.10	15	344.00 > 183.10	21	344.00 > 156.20	30

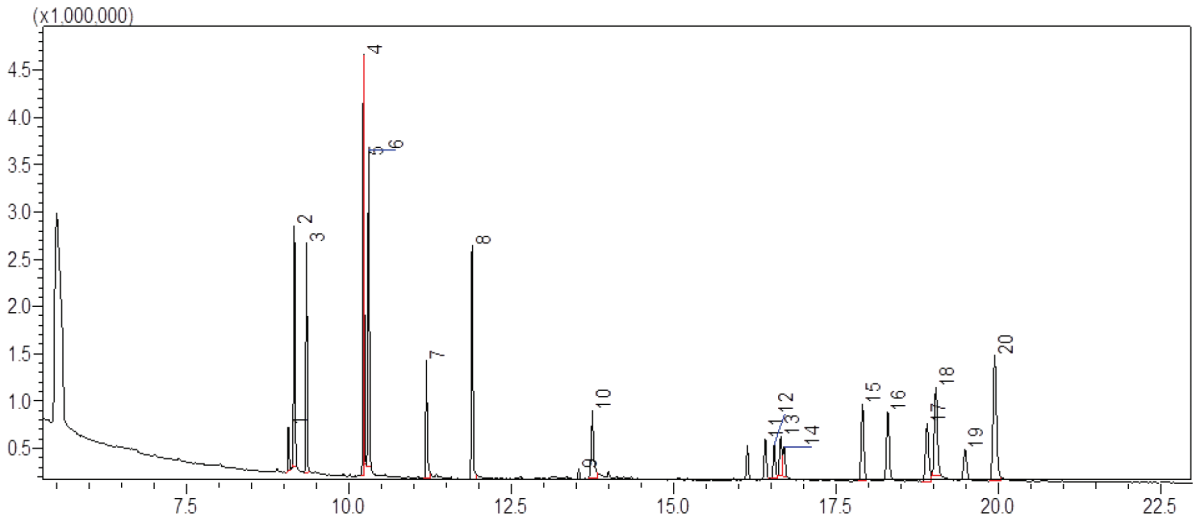

Figure 1. Total ion current chromatogram obtained for GC-MS/MS (SCAN mode) analysis of a pesticide standard solution at 0.1 µg/mL. Peak identities: (1) carbofuran, (2) atrazine, (3) terbufos, (4) alachlor, (5) parathion-methyl, (6) ametrin, (7) thiamethoxam, (8) flutriafol, (9) carbosulfan, (10) acetamiprid, (11) cypermethrin 1, (12) cypermethrin 2, (13) cypermethrin 3, (14) cypermethrin 4, (15) esfenvalerate 1, (16) esfenvalerate 2, (17) difenoconazole 1, (18) difenoconazole 2, (19) deltamethrin, and (20) azoxistrobin.

of pesticide standard solutions. Figure 1 shows a typical chromatogram obtained for a pesticide standard solution at a concentration level of 0.1 µg/mL.

Optimization of the QuEChERS Procedure

Preliminary investigation for optimization of the cleanup step of the QuEChERS procedure for the extraction of pesticides from lager beer was performed using lager beer samples spiked with pesticides at 0.05 µg/mL, and primary and secondary amines (50 mg) and C_{18}-bonded silica (50 mg) as d-SPE sorbents. Acetonitrile was tested as the extracting solvent with a volume of 10 mL. In particular, a good recovery of the selected pesticides was observed when primary and secondary amines (50 mg) and C_{18}-bonded silica (50 mg) as d-SPE sorbents was performed with acetonitrile (24–101 ± 1.8–8.9 %).

Here, the performance of magnesium silicate (50 mg), as a new cleanup sorbent material for d-SPE, was compared with

primary and secondary amines, which were previously tested as the cleanup phase in a multiclass analysis of the same pesticides in lager beer. Recovery experiments were carried out at a fortification level of 0.05 μg/mL. Analyses were performed by GC-MS/MS, with external calibration using matrix-matched standards. Average recoveries ranged from 70 to 93%, with RSD values of 1.9–9.2 %. The values obtained were generally satisfactory, considering the recovery range normally considered acceptable is 70–120%. Consequently, overall results indicate that the combination of magnesium silicate and C_{18}-bonded silica as solid phases is a suitable cleanup procedure for determination of the pesticides in the lager beer matrix (Table 2).

The chromatographic profiles demonstrated the importance of selecting a suitable type of cleanup sorbent in order to remove matrix interferents. There were several peaks in the chromatogram of the control sample, using primary and secondary amines sorbents. A comparison of the cleanup solid-phase sorbents for the determination of the pesticides showed that magnesium silicate gave clean blank chromatograms with minimal interfering peaks from the endogenous components of the sample at the elution time of the pesticides (Figure 2).

Validation of the QuEChERS Method

Once the factors that affect the QuEChERS procedure had been optimized, validation of the method was performed. Recovery values were calculated by comparing the appropriate working standard solutions. Analyses were performed by GC-MS/MS, with external calibration using matrix-matched standards. Using magnesium silicate as d-SPE sorbent, average recoveries ranged from 70 to 123% with RSD values of 0.3–10.5 %. The values obtained were generally satisfactory, considering the recovery range normally considered acceptable (70–120%). The precision and accuracy were considered adequate for validating the method according to the selected criteria. Accuracy was calculated as the percentage ratio between the measured and the known concentrations, and precision was determined as the percentage coefficient of variation (%RSD), which is the ratio between the standard deviation and the mean measured concentration (13).

Table 2. Average recoveries for pesticides for d-SPE tests using blank lager beer samples spiked at 0.05 μg/mL

	Rec. ± RSD % (n = 2)	
Pesticide	PSA	Magnesium silicate
Carbofuran	86 ± 3.9	88 ± 1.9
Atrazine	83 ± 2.4	82 ± 5.7
Terbufos	73 ± 5.5	85 ± 3.9
Alachlor	85 ± 2.8	83 ± 7.1
Parathion-methyl	85 ± 3.1	87 ± 5.4
Ametryn	82 ± 3.0	79 ± 9.2
Thiamethoxam	81 ± 4.7	81 ± 5.4
Flutriafol	84 ± 4.4	76 ± 3.0
Carbosulfan	76 ± 6.1	72 ± 6.1
Acetamiprid	24 ± 5.9	71 ± 5.4
Cypemethrin	83 ± 5.8	78 ± 2.9
Esfenvalerate	81 ± 7.1	70 ± 3.2
Difenoconazole	89 ± 1.8	80 ± 8.0
Deltamethrin	101 ± 8.9	80 ± 7.9
Azoxistrobin	84 ± 1.9	93 ± 5.9

The detector response was linear within the concentration range studied. For all the compounds, the linearity was determined using beer extract fortified at concentration levels of 0.001, 0.005, 0.01, 0.1, 0.25, 0.5, 1.0, 1.5, and 2.5 μg/mL. The slope and intercept values, together with their standard deviations, were determined using regression analyses. The linear regression coefficients ranged from 0.9846 to 0.9937. Matrix effects was determined by the slopes of matrix and solvent calibration curves. Some compounds presented significant (higher than ±20%) matrix effects, in the way this matrix-matched calibration was performed, with analyte solutions prepared in matrix-blank extracts (Table 3).

The LODs were obtained from direct injection of one matrix-matched standard mixture and were calculated based on an S/N ratio of 3; the resulting values ranged from 0.0001 to 0.0007 μg/mL. The LOQs were 0.001–0.006 μg/mL, based on an S/N of 10 (Table 4).

The repeatability of the chromatographic method was determined by replicate analyses of a standard solution at 0.05 μg/mL on different days. The repeatability of the extraction step was evaluated by analyzing seven aliquots of lager beer sample each day over 3 days. The resulting intraday and interday RSD values were below 1.7 and 17%, respectively, which could be considered acceptable, given the difficulty of analyzing these compounds in lager beer.

The focus of this work was to explore the scientific and technological feasibility of application of magnesium silicate material. Economic aspects were not primary concern but are nonetheless important. In this regard, the operational cost of the magnesium silicate was much lower compared with other comercial sorbents, such as primary and secondary amines. The cost of the magnesium silicte was $98 USD/100 g, considerably less than that of comercial PSA ($375 USD/100 g).

Real Sample Analysis

The QuEChERS method developed was applied in determination of the selected pesticides in the nine brands of lager beers obtained from a commercial market of the municipality of Aracaju, Brazil. Pesticide residues were not found in these samples at concentrations above the detection limit.

Conclusions

Magnesium silicate was evaluated as an alternative sorbent in d-SPE step in the application of QuEChERS for determination of acetamiprid, alachlor, ametryn, terbufos, atrazine, azoxystrobin, carbofuran, carbosulfan, cypermethrin, deltamethrin, difenoconazole, esfenvalerate, flutriafol, thiamethoxam, and parathion-methyl residues in lager beer with detection by GC/MS. The results demonstrated that the proposed method provides acceptable accucacy and precision for multiresidue analyses of pesticides. In addition, the method offers considerable savings in terms of cost of materials.

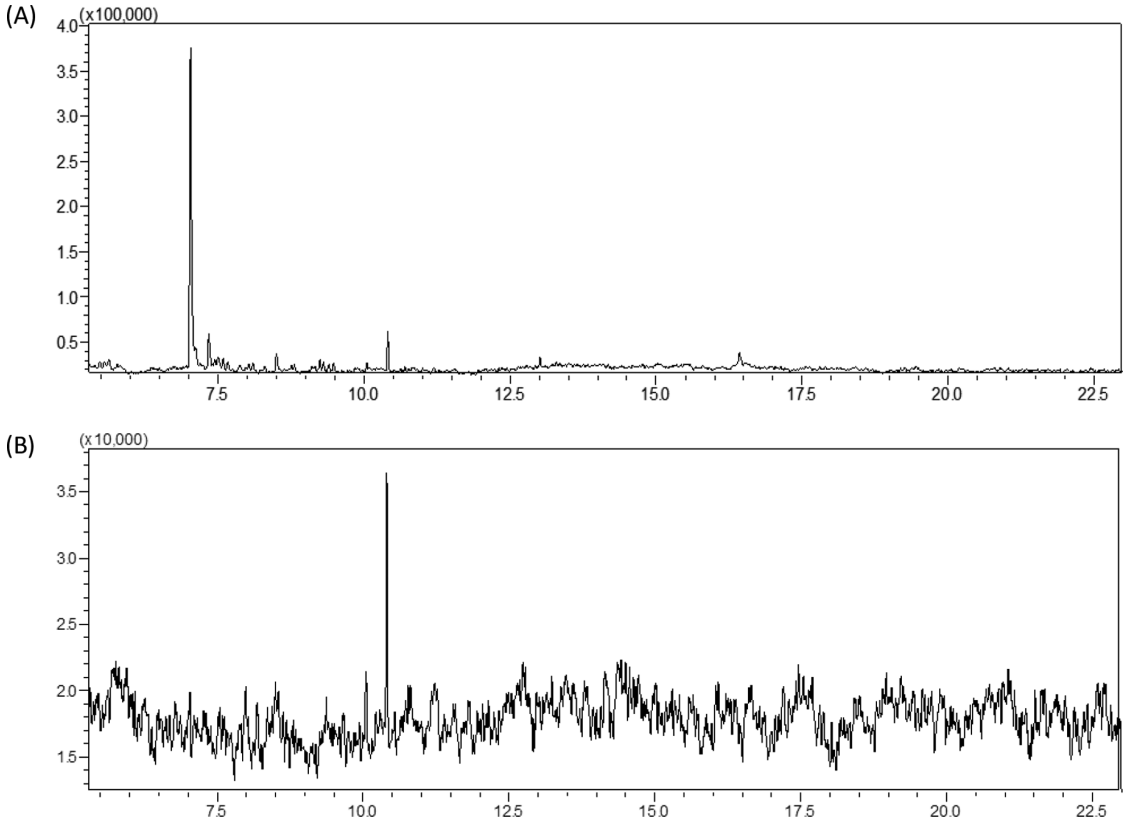

Figure 2. Selective ion chromatograms obtained for GC-MS/MS (MRM mode) analysis of a commercial lager beer sample using primary and secondary amines (A) and magnesium silicate (B) as cleanup adsorbent for QuEChERS procedure. The comparison (difference of detector response) between the chromatograms showed that the proposed adsorbent magnesium silicate was more effective in the removal of interferences from the comercial lager beer than the adsorbent primary and secondary amines.

Table 3. Calibration data and matrix effect for the pesticides analyzed by GC-MS/MS

Pesticide	Linearity range, µg/mL	Equation (matrix)	r^2	Equation (solvent)	r^2	Matrix effect
Carbofuran	0.002–2.5	y = 3316.9x + 98.97	0.9937	y = 3723x – 46.236	0.9985	1.12
Atrazine	0.001–2.5	y = 13000x + 46.277	0.9943	y = 14510x + 37.883	0.9941	1.11
Alachlor	0.001–2.5	y = 39031x + 234.46	0.9984	y = 50267x – 413.23	0.9981	0.77
Terbufos	0.001–2.5	Y= 49141x + 345.45	0.9988	Y=61378x + 524.34	0.9989	0.51
Parathion-methyl	0.001–2.5	y = 10947x – 69.698	0.9804	y = 9505x – 132.55	0.9904	1.15
Ametryn	0.003–2.5	y = 29520x + 79.079	0.9947	y = 35146x – 247.25	0.9917	0.84
Thiamethoxam	0.001–2.5	y = 16059x + 134.86	0.9920	y = 27835x – 461.21	0.9944	0.57
Flutriafol	0.001–2.5	y = 97160x + 106.23	0.9933	y = 87633x – 257.11	0.9970	1.10
Carbosulfan	0.002–2.5	y = 6299.9x – 74.541	0.9906	y = 3416.5x – 46.656	0.9939	1.84
Acetamiprid	0.002–2.5	y = 11308x – 42.327	0.9900	y = 8265.1x – 124.3	0.9958	1.36
Cypermethrin 1	0.002–2.5	y = 7789x – 59.711	0.9910	y = 7065.6x – 86.159	0.9995	1.10
Cypermethrin 2	0.002–2.5	y = 7271.5x – 71.621	0.9903	y = 6843x – 123.15	0.9993	1.06
Cypermethrin 3	0.002–2.5	y = 6189.1x – 57.502	0.9900	y = 5871,3x – 88.492	0.9995	1.05
Cypermethrin 4	0.002–2.5	y = 6571.5x – 87.416	0.9892	y = 6497.9x – 129.72	0.9985	1.01
Esfenvalerate 1	0.002–2.5	y = 6650.2x + 2.1456	0.9846	y = 10815x – 214.78	0.9894	0.61
Esfenvalerate 2	0.002–2.5	y = 6765.9x – 37.584	0.9897	y = 7691.8x – 71.567	0.9979	0.88
Difenoconazole 1	0.004–2.5	y = 13208x – 140.05	0.9808	y = 10971x – 155.72	0.9904	1.20
Difenoconazole 2	0.004–2.5	y = 10092x – 20.47	0.9937	y = 11619x – 212.9	0.9866	0.87
Deltamethrin	0.006–2.5	y = 5541.9x – 27.139	0.9920	y = 6204x – 144.86	0.9912	0.89
Azoxistrobin	0.007–2.5	y = 20695x – 152.04	0.9884	y = 26410x – 508.49	0.9956	0.78

Table 4. Percentage recoveries and relative standard deviations obtained using the QuEChERS procedure applied to lager beer fortified with the pesticides studied

	Rec ± RSD % (*n* = 5)				
Pesticide	0.002, µg/mL	0.01, µg/mL	0.1, µg/mL	LOD, µg/mL	LOQ, µg/mL
Carbofuran	95 ± 3.9	96 ± 10.4	88 ± 10	0.0003	0.002
Atrazine	95 ± 7.7	82 ± 1.9	80 ± 0.3	0.0001	0.001
Terbufos	70 ± 5.2	72 ± 6.4	75 ± 2.4	0.0002	0.001
Alachlor	52 ± 6.0	84 ± 2.2	83 ± 2.9	0.0002	0.001
Parathion-methyl	90 ± 1.6	92 ± 1.9	87 ± 4.5	0.0002	0.001
Ametryn	52 ± 3.8	81 ± 2.5	80 ± 1	0.0009	0.003
Thiamethoxam	77 ± 3.6	83 ± 2.5	82 ± 2.5	0.0003	0.001
Flutriafol	70 ± 6.3	87 ± 2.1	83 ± 1.3	0.0003	0.001
Carbosulfan	20 ± 6.0	37 ± 2.0	43 ± 1.0	0.0002	0.002
Acetamiprid	78 ± 2.3	74 ± 2.0	83 ± 1.8	0.0001	0.002
Cypermethrin 1	79 ± 1.2	76 ± 2.0	123 ± 1.5	0.0003	0.002
Cypermethrin 2	70 ± 10	60 ± 1.5	102 ± 12	0.0003	0.002
Cypermethrin 3	89 ± 4.7	87 ± 1.6	97 ± 1.2	0.0003	0.002
Cypermethrin 4	78 ± 1.5	75 ± 2.7	117 ± 7.7	0.0003	0.002
Esfenvalerate 1	71 ± 2.9	77 ± 8.1	108 ± 2.2	0.0003	0.002
Esfenvalerate 2	84 ± 2.6	74 ± 2.4	112 ± 9.3	0.0003	0.002
Difenoconazole 1	80 ± 1.6	89 ± 2.3	87 ± 7.2	0.0005	0.004
Difenoconazole 2	71 ± 1.7	76 ± 3.9	82 ± 6.4	0.0005	0.004
Deltamethrin	71 ± 3.7	95 ± 7.1	89 ± 6.1	0.0005	0.006
Azoxistrobin	85 ± 6.1	94 ± 3.0	94 ± 10.5	0.0007	0.006

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