Journal of Chromatography A, 1376 (2015) 167–171

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

Analysis of organo-chlorine pesticides residue in raw coffee with a modified "quick easy cheap effective rugged and safe" extraction/clean up procedure for reducing the impact of caffeine on the gas chromatography–mass spectrometry measurement

Bruno Bresin[a], Maria Piol[a], Denis Fabbro[a], Maria Antonietta Mancini[a], Bruno Casetta[b,*], Clorinda Del Bianco[a]

[a] ARPA (Regional Environmental Protection Agency Agenzia Regionale of Friuli Venezia Giulia) – Pordenone Laboratory, Italy
[b] External Mass Spectrometry Consultant p/o ARPA – Pordenone Laboratory, Italy

ARTICLE INFO

Article history:
Received 6 August 2014
Received in revised form
27 November 2014
Accepted 6 December 2014
Available online 12 December 2014

Keywords:
Coffee
QuEChERS
DLLME
Caffeine
Extraction
Pesticides

ABSTRACT

The control of pesticide residues on raw coffee is a task of great importance due to high consumption of this beverage in Italy and in many other countries. High caffeine content can hamper extraction and measurement of any pesticide residue. A tandem extraction protocol has been devised by exploiting the quick easy cheap effective rugged and safe (QuEChERS) scheme for extraction, coupled to a dispersive liquid–liquid micro-extraction (DLLME) in order to drastically reduce caffeine content in the final extract. Gas chromatography–mass spectrometry (GC–MS) has been used for quantification of organo-chlorine pesticides in single ion monitoring (SIM) mode. Method has been validated and performances meet the criteria prescribed by European Union regulations.

1. Introduction

Coffee is one of the most consumed beverages in several countries all over the world and is obtained by *Coffea* ssp. cultivated in tropical regions particularly in Africa, East Asia and South America; compliance of imported raw coffee with European food safety regulations is an emerging issue because there is a need to analyses pesticides residue and other micro contaminants. These determinations are challenged by the particularity of coffee that contains huge amount of caffeine.

There are few papers dealing with pesticide residues analysis in raw coffee: one work use ethyl acetate for extraction and gel permeation chromatography for purification [1], while others [2–4] uses QuEChERS approach. So far none of these papers addresses the high caffeine content of extracts.

QuEChERS is a well-established method for extraction of pesticide residues in fruit and vegetables [5,6] and some improvements and widening of the scope were made until today [7]. It is suitable for raw coffee but the coextracted caffeine represents a problem when GC–MS is the analytical technique employed: the large amount of caffeine injected is problematic for the integrity of the chromatographic system, quantitative analysis is worsened in the region of caffeine elution and qualitative analysis is hampered by the very large and broad peak of caffeine.

Dispersive liquid–liquid microextraction (DLLME) is a technique developed by Rezaee and coworkers [8] for preconcentration of lipophilic analytes from aqueous matrices: it consists on an extraction performed by little amount of an organic solvent immiscible with water dispersed in aqueous sample by another solvent miscible with water. The result is a cloudy mixture with an extremely dispersed extraction solvent which is successively separated by centrifugation. In these years DLLME has showed a rapid growth: it was employed for analysis of organophosphorous pesticides in water [9], coupled with solid phase extraction in determination of chlorophenols in aqueous samples [10], used for simultaneous complexation and extraction of metals from water [11] and simultaneous derivatization and extraction of chlorophenols from water [12]. New applications such as ionic liquid DLLME [13] and RP-DLLME [14] has been implemented.

* Corresponding author. Tel.: +39 04321918114, +39 3348265346.
E-mail address: bruno.casetta@yahoo.com (B. Casetta).

http://dx.doi.org/10.1016/j.chroma.2014.12.016
0021-9673/

Development and application of DLLME has been recently reviewed [15,16].

We have devised a reverse mode for DLLME: the acetonitrile QuEChERS extract is the disperser solvent, carbon disulfide is the extraction solvent and deionized water is added to form a ternary mixture. By this method organo-chlorine pesticides are efficiently concentrated in carbon disulfide and a large portion of caffeine remains in the water–acetonitrile phase.

2. Experimental

2.1. Chemicals and materials

Mix of organo-chlorine pesticides at 10 μg/mL in acetonitrile and triphenyl-phosphate at 1000 μg/mL in acetonitrile were from Neochema.

Acetonitrile and formic acid 98% were from Carlo Erba. Anhydrous magnesium sulfate, sodium chloride, sodium citrate tribasic dihydrate, sodium citrate dibasic sesquihydrate were from Sigma.

Analytes protectants (D-sorbitol, δ-gulonolactone, shikimic acid, 3-ethoxy-1,2-propandiol) were all from Sigma. Protectants mixture solution was made by dissolving 0.015 g of D-sorbitol and 0.03 g of δ-gulonolactone in 0.5 mL of water, by adding subsequently 0.6 g of 3-ethoxy-1,2-propandiol and 0.015 g of shikimic acid. After adding 0.05 mL of formic acid, resulting solution was filled up to 100 mL with acetonitrile. This solution was used to prepare working standard solutions and to re-dissolve sample extract before the GC–MS injection.

Triphenyl-phosphate was diluted to 50 μg/mL with acetonitrile and working standard solutions were prepared by dilutions with the protectants mixture solution to obtain 0.02, 0.05, 0.2 and 0.5 μg/mL of organo-chlorine pesticide solutions, each containing 0.5 μg/mL of internal standard triphenyl-phosphate.

Pre-packaged 2-mL plastic centrifuge tubes containing 150 mg anhydrous magnesium sulfate, 25 mg PSA (primary secondary amine resin) and 25 mg C18 were from Restek.

Deionized water was supplied by the in-house milliQ Millipore equipment.

2.2. Sample pretreatment

Collected samples of raw coffee in grains were stored overnight at −80 °C. This freezing treatment was necessary to make successful the subsequent milling. The sample was milled when still at that low temperature by a laboratory miller in order to obtain a fine powder.

2.3. Extraction

2 g of milled sample were weighed in a 50 mL polypropylene tube and 10 mL of milliQ water were added. After 15 min at 8 °C for the complete hydration of the sample, 10 mL of acetonitrile and 20 μL of internal standard (triphenyl-phosphate, 50 μg/mL in acetonitrile) were added.

The sample was vigorously shaken for 1 min: a mixture of pre-weighted salts was added (4 g anhydrous magnesium sulfate, 1 g sodium chloride, 1 g sodium citrate tribasic dihydrate, 0.5 g sodium citrate dibasic sesqui-hydrate) and mixture was again shaken for 1 min. After 5 min of centrifugation at 3500 rpm the upper acetonitrile phase was transferred in another 15-mL polypropylene tube and left at −18 °C overnight. By this freeze-out treatment, large part of lipids and waxes was made precipitated since un-dissolved for the low temperature.

2.4. Clean up and DLLME

Centrifuge tube with 150 mg anhydrous magnesium sulfate, 25 mg PSA and 25 mg C18 was filled with 1 mL of acetonitrile extract as coming from the freeze-out step, shaken for 30 s and centrifuged at 3500 rpm for 5 min.

100 μL of carbon disulfide were dissolved in 0.5 mL of acetonitrile in a glass centrifuge test tube; 0.5 mL of the acetonitrile-extract purified with PSA and C18 were added and mixed. By an automatic pipette 5 mL of deionized water were rapidly added and a cloudy solution was immediately formed. The tube was capped and mixed by turning upside down for 5 times; and centrifuged at 5000 rpm for 4 min. The upper aqueous solution was discarded by a Pasteur pipette; carbon disulfide layer staying at the bottom of test tube was dried in a vacuum centrifuge at 40 °C. Residue was re-dissolved with 100 μL of protectants mixture solution and transferred to a vial for GC–MS analysis. This solution corresponds to 1 g/mL of coffee.

2.5. Instrumental analysis

For GC–MS, an Agilent (Wilmington, DE, USA) 6890 GC equipped with split–splitless injector and autosampler coupled to a 5973i mass spectrometer (single quadrupole) was used. A 5 m, 0.25 mm i.d., guard column was coupled by a press-fit, to the 30 m, 0.25 mm i.d., 0.25 μm film thickness Agilent HP5 MS UI analytical column and, by another press-fit, column was coupled to a 30 cm-transfer line which enters the MS interface.

Splitless injection of the final extracts and standards was made into a deactivated glass liner filled with glass wool; 1 μL was injected for quantitative analysis in SIM mode and 2 μL for qualitative analysis in scan mode. Injector was held at 250 °C and

Table 1
Pesticides measured by GC–MS.

Analytes	Retention times (min)	Target ions	Qualifier ions
alpha-HCH	12.03	181.0	218.9, 183.0, 216.9
beta-HCH	13.22	219.0	181.0, 183.0, 217.0
gamma-HCH (Lindane)	13.40	181.0	183.0, 218.9, 216.9
Heptachlor	16.73	271.8	273.8, 269.8
Aldrin	18.46	262.9	264.9, 260.9, 66.1
Heptachlor endo-epoxide (trans)	20.93	217.0	184.9, 252.9, 182.9
alpha-Endosulfan	22.60	236.9	195.0, 238.9, 240.9
Dieldrin	23.84	262.9	276.9, 278.9
p,p′-DDE	24.01	246.0	318.0, 316.0, 248.0
Endrin	24.74	262.9	264.9, 260.9, 81.1
beta-Endosulfan	25.15	195.0	236.9, 197.0, 207.0
p,p′-DDD	25.70	235.0	237.0, 165.1, 236.0
o,p′-DDT	25.78	235.0	237.0, 165.1, 199.1
Endosulfan sulfate	26.77	272.0	274.0, 229.0, 237.0
p,p′-DDT	27.00	235.0	237.0, 165.1, 236.1
Triphenylphosphate IS	27.76	326.1	325.1
Methoxychlor	28.89	227.0	228.0, 152.0, 113.0