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Planar solid phase extraction—A new clean-up concept in multi-residue analysis of pesticides by liquid chromatography–mass spectrometry

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ABSTRACT

Efficient clean-up is indispensable for preventing matrix effects in multi-residue analysis of pesticides in food by liquid and gas chromatography coupled to mass spectrometry. As a completely new approach, highly automated planar chromatographic tools were applied for powerful clean-up, called high-throughput planar solid phase extraction (HTpSPE). Thin-layer chromatography (TLC) was used to completely separate pesticides from matrix compounds and to focus them into a sharp zone, followed by extraction of the target zone by the TLC–MS interface. HTpSPE resulted in extracts nearly free of interference and free of matrix effects, as shown for seven chemically representative pesticides in four different matrices (apples, cucumbers, red grapes, tomatoes). Regarding the clean-up step, quantification by LC–MS provided mean recovery (against solvent standards) of 90–104% with relative standard deviations of 0.3–4.1% ($n = 5$) for two spiking levels of 0.1 and 0.5 mg/kg. Clean-up of one sample was completed in a manner of minutes, while running numerous samples in parallel at reduced costs, with very low sample and solvent volumes.

1. Introduction

Pesticides are widely used in agriculture during the cultivation and storage of fruits and vegetables to ensure quality and food safety. Besides their positive effects, they may be harmful to human health, depending on the level of residues. Therefore, most countries have laid down strict regulations concerning pesticide residues. In the European Union (EU), maximum residue limits (MRLs) are regulated by the European Union Council Directive 91/414/EEC [1] for over 500 pesticides in food and feed. Consequently, sensitive, selective and robust analytical techniques for pesticide residues analysis are required.

Different than traditional detector systems, liquid chromatography (LC) and gas chromatography (GC) coupled to mass spectrometry (MS) provide a high degree of selectivity and sensitivity. During the early years of LC–MS applications in residue analysis, analysts proposed the LC column of a LC–MS system as nearly dispensable, while the separation was performed by the mass spectrometer. Meanwhile, so-called "matrix effects" have been recognised as one of the major sources of uncertainty in LC–MS [2,3], which have also been called the "Achilles heel" [3] of quantitative trace analysis by LC–MS. Co-eluting matrix components may result in (i) false negatives, (ii) false positives, or (iii) inexact quantification caused by ion suppression or ion enhancement, depending on the matrix [3]. The same problems hold true for GC–MS, but different matrix compounds interfere during LC–MS and GC–MS, and different mechanisms are responsible for these matrix effects [2,4].

To overcome the problems of matrix effects, different clean-up methods by using gel permeation chromatography (GPC), cartridge solid phase extraction (SPE) or dispersive solid phase extraction (dSPE) [5] on different materials have been proposed to remove fatty acids, lipids, phenols, chlorophyll, and other co-extracted matrix compounds from the extracts of fruits and vegetables [6–10]. However, these methods are partly sensitive to losing pesticides [11], which is why several compromises have to be made. Additionally, these methods are usually time consuming, costly, and almost all involve the use of a large amounts of organic solvents. Therefore, the present study focused on the development of a feasible, easy and rapid planar chromatographic clean-up method for the separation of pesticides from matrix compounds followed by LC–MS analysis.

High performance thin-layer chromatography (HPTLC) is a widely used technique for qualitative and quantitative analysis in

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diverse fields such as the environmental, food, and pharmaceutical sciences [12]. The ability to detect nearly everything on the plate, combined with fast side-by-side sample analysis under repeatable conditions, high automation and the capability of multi-detection represent some of the advantages of HPTLC [13]. We used these benefits to introduce a new technique for clean-up in pesticide residue analysis on planar thin layers, replacing SPE and GPC. In keeping with the concept of a fast, reliable and highly reproducible clean-up method, automated sample application and plate development as well as TLC extraction tools are integral parts of the method.

2. Materials and methods

2.1. Chemicals and materials

Acetamiprid, penconazole, and the internal standard (ISTD) tris(1,3-dichloro-2-propyl)phosphate (TDCPP) were purchased from High Purity Compounds (Cunnersdorf, Germany), azoxystrobin, fenarimol and mepanipyrim from Dr. Ehrenstorfer (Augsburg, Germany), and chlorpyrifos, pirimicarb and Sudan II from Fluka–Sigma–Aldrich (Steinheim, Germany). Oleic acid (>60%) was from Merck (Darmstadt, Germany) and soy bean oil from a local supermarket. Primuline for postchromatographic derivatisation of TLC plates was purchased from Waldeck (Münster, Germany). Bondesil–PSA (primary secondary amine), 40 μm was obtained from Varian Inc. (Palo Alto, USA). Acetone (Rotisolv pestilyse) was purchased from Carl Roth GmbH+ Co. KG (Karlsruhe, Germany). Acetonitrile (gradient grade), methanol (LC–MS Chromasolv), formic acid (analytical reagent grade) and ammonium formate (>97.0%) were obtained from Sigma–Aldrich (Steinheim, Germany). TLC aluminium foil silica gel 60 NH_2 F_{254}s, 20 cm × 20 cm, with a layer thickness of 0.15–0.18 mm from Merck (Darmstadt, Germany) were prewashed with acetonitrile, dried in an oven at 100 °C for 15 min, and stored in a desiccator until use. For preliminary experiments, TLC plates and foils were obtained from Merck (Darmstadt, Germany) and Machery-Nagel (Düren, Germany).

2.2. Solutions

A standard stock solution containing seven pesticides at a concentration of 10 μg/mL was prepared in acetonitrile. The internal standard TDCPP and Sudan II were dissolved in acetonitrile at a concentration of 250 and 100 μg/mL, respectively. The stock solutions were stored at −19 °C.

Spiking solutions were prepared by diluting stock solutions with acetonitrile, resulting in concentration of 5 μg/mL pesticides, 50 μg/mL TDCPP and 10 μg/mL Sudan II (for the 0.5 mg/kg spiking level), and 1 μg/mL pesticides, 10 μg/mL TDCPP and 10 μg/mL Sudan II, respectively (for the 0.1 mg/kg spiking level).

2.3. TLC instrumentation

An Automatic TLC Sampler 4 (ATS 4, CAMAG, Muttenz, Switzerland) was used to apply samples (50 μL, 3.0 mm × 4.0 mm areas). Acetonitrile was used as rinsing solvent. Application parameters (predefined for methanol) were set to: filling speed 13 μL/s, predosage volume 300 nL, retraction volume 200 nL, dosage speed 290 nL/s, rinsing vacuum time 5 s, filling vaccum time 1 s, rinsing cycles 1, filling cycles 1. The following application settings were used, leading to 10 tracks on a 10 cm × 10 cm foil: 13 mm distance from the lower edge, 16.5 mm distance from the left edge, and 8.5 mm track distance. Chromatography was performed in the Automatic Developing Chamber (ADC2, CAMAG) with a 20 cm × 10 cm twin-through chamber (CAMAG). For plate image documentation, the DigiStore 2 Documentation System (CAMAG) consisting of a Reprostar 3 illuminator with a Baumer Optronic DXA252 digital camera was used with the following settings: 60 ms exposure time (visible range), 130 ms (254 nm), and 3000 ms (366 nm) at a gain of 1. The automatic background correction was used to increase performance. A TLC Immersion Device (CAMAG) was used with an immersion speed of 2 cm/s and an immersion time of 0 s. TLC instruments were controlled by WinCats 1.4.2 Planar Chromatography Manager (CAMAG).

The zone extraction instrument consisted of the TLC–MS Interface (CAMAG), equipped with a circular extraction head (4 mm) plunger operated by a separate pump (WellChrom K-1000 MaxiStar, isocratic pump, Knauer GmbH, Berlin, Germany).

2.4. LC–MS analysis

The LC–MS equipment consisted of an Agilent 1100 modular HPLC system with a quaternary pump, vacuum solvent degasser unit, column oven, and autosampler, coupled to a G1956B MSD single quadrupole mass spectrometer equipped with an electro spray (ESI) interface, and was operated by ChemStation B.02.01 SR2 software (Agilent Technologies Deutschland GmbH, Waldbronn, Germany). Chromatography was performed at 40 °C on a Chromolith Performance RP-18 endcapped, 100 mm × 3.0 mm column with the corresponding 5 mm × 3 mm guard column (Merck, Darmstadt, Germany). Mobile phase A was acetonitrile and mobile phase B was 10 mM ammonium formate. Gradient elution started with 10% A (0–1 min), linearly increased to 45% A (1–2 min), then linearly to 55% (2–7 min) and to 90% (7–9 min), kept constant at 90% (9–13 min), then decreased to 10% in 0.2 min and held for 5 min to equilibrate the column. The injection volume was generally 12 μL. The mass spectrometer operated under the following parameters for positive electrospray ionisation: capillary voltage 4.0 kV, skimmer voltage 35 V, lens 2.5 V, quadrupole temperature 100 °C, drying gas temperature 300 °C, drying gas flow rate 10 L/min and nebuliser gas pressure 40 psig. Total ion chromatograms (TIC) in the positive scan mode were recorded from m/z 100 to 600 using a fragmentor voltage of 100 V, gain 1, threshold 100, and step size 0.25. Quantification was performed in selected ion monitoring (SIM) mode at m/z 223.1, 239.1, 331, 404.1, 224.1, 284, 430.8, 447.8, 349.9 and 277.1 for acetamiprid, primicarb, fenarimol, azoxystrobin, mepanipyrim, penconazole, TDCPP, chlorpyrifos, and Sudan II, respectively, whereas seven time windows were used. Additional settings were a fragmentor voltage of 100 V, gain 5, threshold 100, and step size 0.25.

2.5. Samples and extraction

Apples, red grapes, tomatoes and cucumbers were used as representative fruit and vegetable matrices. They were organically produced and obtained from local supermarkets. The QuEChERS-method [5] was used as a guideline for sample extraction. Samples were cut into pieces and ground by a GRINDOMIX GM 300 knife mill (Retsch GmbH, Haan, Germany). For centrifugation, a Biofuge primo R (Hereaus GmbH, Hanau, Germany) was used.

Spiked extracts were prepared by a 10-fold dilution of the spiking solution with the raw extract (post-extraction addition). Corresponding standard solutions were prepared in the same way using acetonitrile as the diluting agent. The final concentrations in the measuring solution for LC–MS were 0.125 μg/mL pesticides, 1.25 μg/mL TDCPP, and 0.25 μg/mL Sudan II (for the 0.5 mg/kg spiking level), and 0.025 μg/mL, 0.25 μg/mL, and 0.25 μg/mL, respectively (for the spiking level of 0.1 mg/kg). For direct measurement without clean-up, the spiked extracts were diluted 1:4 with acetonitrile/10 mM ammonium formate (1/2, v/v).

2.6. Clean-up methods

2.6.1. dSPE clean-up with PSA

dSPE clean-up with PSA was done with 2 mL of each extract according to the QuEChERS-method [5]. Before LC–MS analysis, dSPE extracts were diluted 1:4 with acetonitrile/10 mM ammonium formate (1/2, v/v).

2.6.2. HTpSPE clean-up

Before application, TLC aluminium foil silica gel 60 NH_2 F_{254}s (10 cm × 10 cm) were dipped 20 mm deep in a 2% formic acid solution in acetonitrile and dried in a stream of warm air for 10 min. After the application of extracts (50 μL), the start zones were dried in a warm air stream for 5 min, and chromatography was performed in the ADC2 using acetonitrile as the mobile phase up to a migration distance of 75 mm. Drying in a stream of cold air followed for 5 min. A second development with acetone was carried out in the backwards direction to a migration distance of 46 mm. The target analyte zone of each track, visible by Sudan II dye, was extracted by the TLC–MS interface into autosampler vials and measured by LC–MS. As the extraction solvent, acetonitrile/10 mM ammonium formate (1/1, v/v) was used at a flow rate of 0.2 mL/min for 60 s, leading to 200 μL extract from a single sample zone. Blank extracts were prepared identically.

2.7. Determination of matrix and matrix effects

2.7.1. Determination of matrix by digital documentation of TLC foils

Raw extracts, extracts after dSPE with PSA, and extracts after HTpSPE clean-up (all extracts with a concentration of 1 g sample/mL) were applied onto TLC aluminium foil silica gel 60 NH_2 F_{254}s, 50 μL each. Chromatographic development was done using acetonitrile as the mobile phase up to a migration distance of 75 mm. As comparison standards, oleic acid and soy bean oil (0.1% in acetone, 5 μL) were applied. Documentation was performed under UV illumination at 254 and 366 nm, and in the visible range (reflectance mode). For derivatisation, the developed TLC foils were dipped into a solution of 0.05% primuline in acetone–water (4 + 1, v/v), dried in a stream of warm air, and documented under 366 nm.

2.7.2. Determination of matrix by LC–MS

Blank extracts as well as extracts spiked with a pesticide mixture at 0.5 mg/kg were prepared for each matrix (raw extracts, extracts after dSPE, and extracts after HTpSPE), and total ion chromatograms (TIC) were recorded. A standard pesticide mixture at a corresponding concentration was additionally measured. Efficiency of clean-up was determined by comparison of the number, intensity and region of detected interfering matrix compound peaks.

2.7.3. Determination of matrix effects by recovery experiments

"Matrix effects" were evaluated by comparing the responses of a pesticide in a pure standard solution to those of a spiked matrix extract at the same concentration (both normalised to the TDCPP internal standard, using peak areas). dSPE and HTpSPE clean-up was done according the procedures listed above. Spiked samples from each of the four matrices were examined at 0.1 and 0.5 mg/kg spiking level. LC–MS was performed in SIM mode.

3. Results and discussion

3.1. Approach

Thin-layer chromatography (TLC) was used to develop a new clean-up technique for QuEChERS [5] extracts, called high-throughput planar solid phase extraction (HTpSPE). Three sets of experiments were performed. In the first set, TLC clean-up and TLC zone extraction methodologies were tested and optimised. In the second step, matrix co-extractives were identified by digital imaging of TLC foils as well as by comparing and assessing LC–MS total ion chromatograms of blank and spiked sample extracts to check for interference in the chromatograms. In the third step, recovery and relative standard deviations were determined with four different sample matrices at spiking levels of 0.1 and 0.5 mg/kg by LC–MS, when HTpSPE was compared to the primary secondary amine (PSA) dSPE [5].

3.2. HTpSPE

With the aim of leaving matrix compounds behind and to collect pesticides into one sharp zone, different TLC materials including normal phase silica, C18 reversed phase silica, and aminopropyl modified silica were tested using a wide range of solvents according to the different selectivity of groups referred to Snyder [14] (data not shown). Additionally, several developing strategies were examined, such as single and multiple development in the same direction or backwards direction, or cutting the lower TLC plate (with matrix compounds) off before a second development. Due to the numerous detection possibilities in planar chromatography, the clean-up result was directly visible. For evaluation, digital documentation of TLC plates was performed by UV illumination both at 254 nm and 366 nm, under visible light, and after derivatisation with primuline to sensitively detect lipids.

As the result of the screening studies, an amino-modified silica foil was identified as giving the best results, which is in accordance with the PSA dSPE clean-up of the QuEChERS method. A twofold development was chosen, first with acetonitrile up to a migration distance of 75 mm, and second after 180° rotation with acetone to a migration distance of 46 mm (Fig. 1). On a 20 cm × 10 cm foil, twenty samples were simultaneously cleaned-up with 10 mL portions of each mobile phase. Thus, the solvent consumption was 1 mL per sample and the run time was 50 s per sample. With the automatic sample application as the most time-consuming step, the total clean-up took about 70 min for 20 samples, resulting in an overall clean-up time of 3.5 min per sample.

The target analyte zones of pesticides were made visible by the addition of Sudan II as a marker to the sample extracts, which thereafter were extracted by the TLC–MS interface into autosampler vials (Fig. 2). Different solvents/solvent mixtures were tested for the extraction of pesticides from the TLC zone, while flow rates and extractions times were modified. Optimal recovery was obtained with acetonitrile/10 mM ammonium formate (1/1, v/v) at a flow rate of 0.2 mL/min for an extraction time of 60 s.

3.3. Assessment of clean-up efficiency

QuEChERS extracts were prepared from tomatoes, cucumbers, apples and grapes, and spiked with a pesticide mixture containing seven pesticides, representing a wide spectrum of polarity and different substance classes (Table 1S). For clean-up experiments, HTpSPE and dispersive SPE with PSA were compared. Visible inspection of the extracts already showed great differences in colour and colour intensity between the two clean-up methods. However, since most matrix compounds are not directly visible, clean-up efficiency was assessed through different methods. First, the TLC separated matrix load was detected using an image capture device under different illuminations and after primuline derivatisation. Successful HTpSPE clean-up was visible at once (Fig. 1). Nearly the complete matrix load from tomatoes, cucumbers, apples and grapes, detected under UV 366 nm and white light (similarly under UV 254 nm, not shown) was separated from the target analyte zone in which pesticide residues were focused. Organic acids, sugars, phenols, and other matrix components remained at

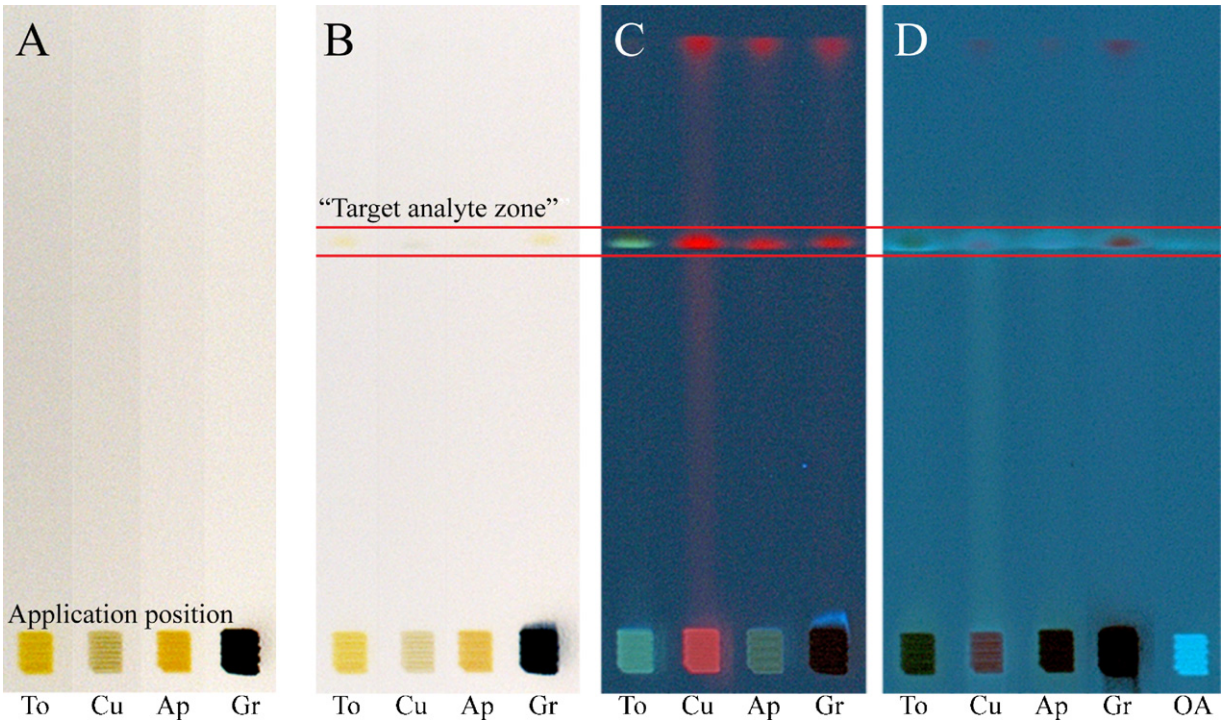

Fig. 1. Separation of raw QuEChERS extracts of tomatoes (To), cucumber (Cu), apples (Ap) and red grapes (Gr) on TLC aluminium foil silica gel 60 NH_2 F_{254}s: before development under white light (A), after twofold development (HTpSPE) under white light (B), UV 366 nm (C) and UV 366 nm after derivatisation with primuline (D). Oleic acid (OA) was applied as a fatty acid exemplar.

the application position. After derivatisation with primuline, fatty acids and other lipophilic substances such as phospholipids and triglycerides were visible under UV 366 nm. Fatty acids, which cause prominent matrix effects especially during GC–MS analyses, were completely removed by HTpSPE, as shown using the example of an oleic acid standard.

Raw extracts, extracts after PSA dSPE, and extracts after HTpSPE were also compared by TLC using the same chromatographic system (Fig. 3). Raw extracts and PSA dSPE extracts showed no considerable differences concerning the matrix load. After primuline derivatisation, it became apparent that dSPE clean-up only slightly removed fatty acids, especially in apple (track 3 vs. 4) and tomato (track 7 vs. 8) extracts. Both raw and PSA cleaned-up extracts showed intensive zones of fatty acids and other matrix components at the start position (track 1: oleic acid for comparison). In comparison, HTpSPE resulted in excellent clean-up (tracks 11, 12, 13, 14); no matrix components were detected at UV 254 nm and under white light, either at the application position or on the entire developed track. HTpSPE removes fatty acids completely and reduces triglyceride components, located in the solvent front (track 2: soy bean oil for comparison). This reduction was caused by the polarity of the extraction solvent during the TLC extraction procedure.

Another way to compare and assess the clean-up effect was to record LC–MS total ion chromatograms (TICs) of the extracts. The inspection of TICs of both un-spiked and spiked tomato extracts (Fig. 4) supported the results obtained by digital documentation of TLC foils. Blank raw extracts revealed many interfering signals across the whole chromatogram. Particularly at retention times of about 3 min and between 7 and 9 min (polar components), and from 13 min on up to the end (less polar compounds), an immense matrix load was evident. On the one hand, distinct chromatographic peaks were observed (e.g. at 3.3, 8.4 or 14.3 min) indicating the elution of a single matrix component. On the other hand, broad humps occurred (e.g. between 7 and 9 min) as a sign of either overloading by matrix compounds or of chemically diverse, chromatographically badly resolved matrix components. Compared with extracts obtained after dSPE with PSA, the matrix signals were reduced only marginally (Fig. 4). Contrarily, the successful HTpSPE clean-up was clearly visible; no interfering signals were detected up to 15 min, and considerably fewer and less intense ones between 15 and 18 min. This indicates that HTpSPE removed both polar and

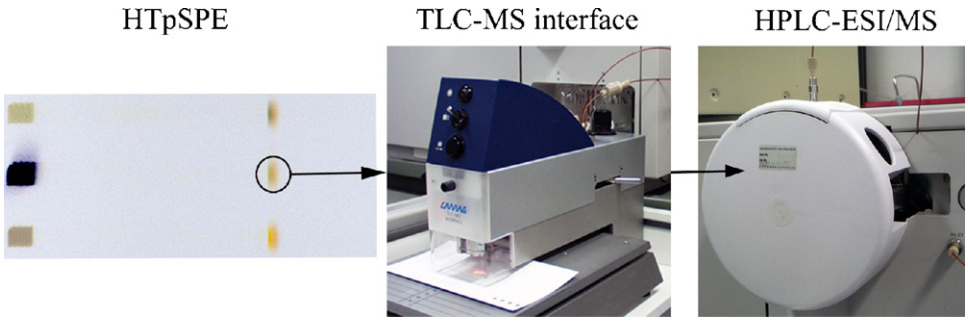

Fig. 2. Extraction procedure of a target analyte spot by TLC–MS interface followed by HPLC–ESI/MS analysis.

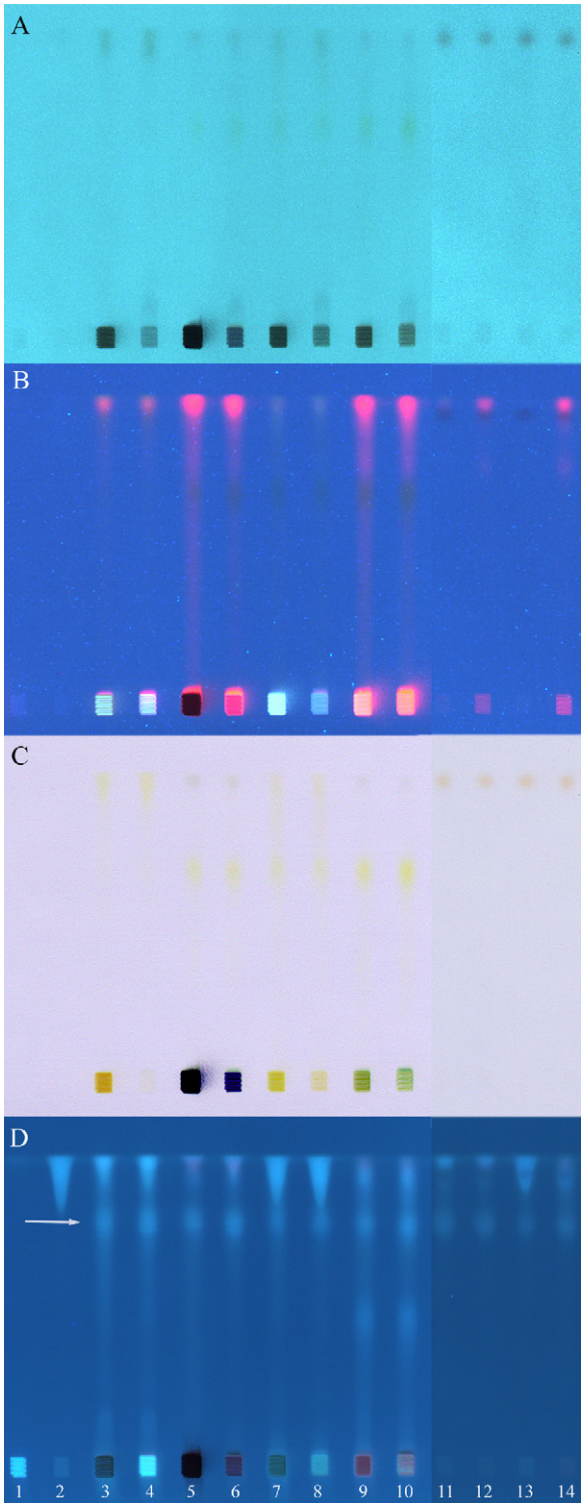

Fig. 3. Comparison of clean-up effect for four different matrices (apples: 3, 4, 11; grapes: 5, 6, 12; tomatoes: 7, 8, 13; and cucumbers: 9, 10, 14); raw QuEChERS extracts (3, 5, 7, 9), after dSPE clean-up with PSA (4, 6, 8, 10), and after HTpSPE clean-up (11, 12, 13, 14). Separation on TLC aluminium foil silica gel 60 NH_2 F_{254}s using acetonitrile as the mobile phase up to a migration distance of 75 mm. Documentation of the developed foils under UV 254 nm (A), 366 nm (B), white light (C), and after derivatisation with primuline at 366 nm (D). Oleic acid (1) and soy bean oil (2) were applied to show the behaviour of fatty acids and fats, respectively. The arrow marks a contaminant migrating from the polypropylene tubes.

non-polar matrix components. The TICs of extracts obtained after HTpSPE were very similar for all the studied commodities (Fig. 1S), which demonstrated the potential clean-up of HTpSPE independent of matrices of different compositions.

Tomato extracts, for example, spiked at 0.5 mg/kg showed that nearly all pesticides co-eluted with co-extracted matrix components, which was observed both in raw sample extracts and in sample extracts after common dSPE with PSA (Fig. 4). Since HTpSPE clean-up on the other hand revealed almost no or less intensive interfering co-extracted matrix signals, the chromatograms were nearly identical with that of a standard mixture of pesticides. Thus, a neat solvent standard can simply be used for calibration instead of matrix-matched standards. The same clean-up results were obtained for cucumber extracts, showing differences in interfering signals concerning retention time and the intensity of detected co-extractives (Fig. 1S). In apple and grape extracts (Fig. 1S), matrix load was generally lower; nevertheless, the efficiency of HTpSPE was evident.

After HTpSPE, spiked extracts showed an additional peak at 10.5 min, while the peak area of azoxystrobin (R_t = 11.4 min) decreased (Fig. 4B, lane c), when compared to a standard pesticide mixture. The reason was an *E*/*Z* isomerisation of azoxystrobin, probably during HTpSPE, but also known from phototransformation in solutions [15], and also for several other pesticides after dSPE with PSA. However, in the case of azoxystrobin the two peaks of the stereoisomers were simply summed up for quantification.

3.4. Recovery studies

Recovery using the QuEChERS method has been extensively published [5,16,17]. In this study, recovery was only determined concerning the clean-up method. Therefore, blank extracts were spiked with a pesticide mixture, followed by PSA dSPE or HTpSPE clean-up, and analysed by LC–MS. For HTpSPE, the studied pesticides generally gave exceptionally good results, except for chlorpyrifos. This pesticide was surprisingly problematic during HTpSPE, resulting in average recovery of 140–234% and relative standard deviations (RSD) ranging between 2.6 and 12.8% (n = 5) in four matrices at two spiking levels (0.1 mg/kg and 0.5 mg/kg) (Table 2S). As already known in pesticide residue analysis, quite a few pesticides are base-sensitive during PSA clean-up, like chlorothalonil, folpet or acephate, while others are prone to oxidation, like fenthion, fenamiphos or methiocarb [18], but to the best of our knowledge there is no comment in the literature concerning the instability of chlorpyrifos. However, chlorpyrifos seemed to degrade upon contact with the active amino groups of the modified silica planar layers, although this degradation was only observed for the pure solvent standard (applied onto the plate for calibration), not in the presence of sample matrix on the plate. As fruit and vegetable matrices contain defined amounts of fruit acids, they obviously protect chlorpyrifos applied onto basic amino modified silica thin layers, while standard solutions were free of acids. According to the increasing amounts of fruit acids in cucumber < grapes ≈ tomatoes < apples [19], the corresponding overall mean recovery (n = 5, at both spiking levels) increased in the same order 145% < 165% ≈ 171% < 204% (Table 2S). Additionally, RSD values of 2.6–12.8% at different spiking levels and in different matrices were relatively high. These findings strongly confirm the assumption that fruit acids present in the extract are responsible for chlorpyrifos stabilisation. Therefore, several approaches to buffer the active amino groups of the silica layer were tested to obtain maximum recovery from standard solutions. Additional aspects were repeatability, reliability, time consumption and handling of the procedure. The performed experiments included the addition of formic acid to the standard solutions (0.05–0.5%) and the addition of formic acid to the mobile phase used for HTpSPE

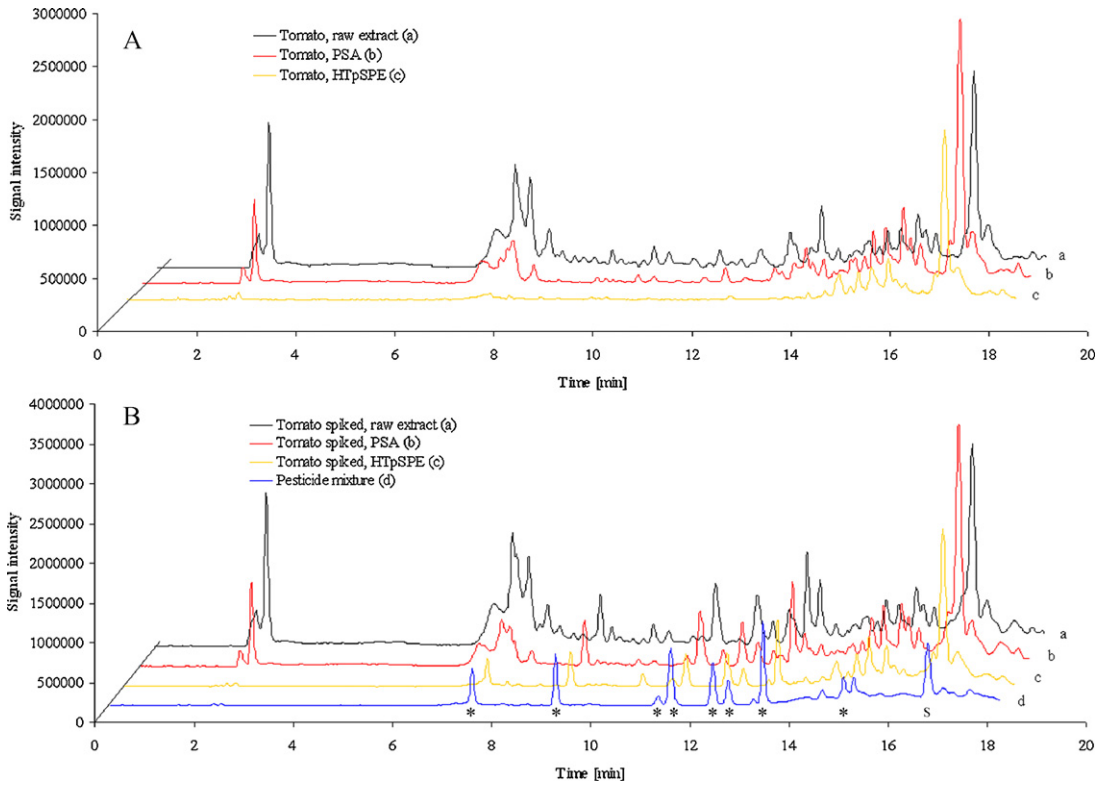

Fig. 4. Comparison of LC–MS total ion chromatograms of tomato blank extracts (A) and extracts spiked with a pesticide mixture at a level of 0.5 mg/kg (B), using different clean-up methods; QuEChERS raw extract (a), after dSPE clean-up with PSA (b), and after HTpSPE (c). Track d refers to a pesticide (*) standard mixture of acetamiprid, pirimicarb, fenarimol, azoxystrobin, mepanipyrim, penconazole, TDCPP, and chlorpyrifos (from left to right), and Sudan II (s).

(0.2–2%). Furthermore, ammonium formate (pH 4) was added to standard solutions and sample extracts to final concentrations of 1–5 mM. Another approach was dipping the application zone of TLC foils into a solution of formic acid in acetonitrile (0.5–5%) before the sample application was done, which finally was the most efficient procedure in terms of chlorpyrifos recovery; a 2% solution of formic acid was appropriate. In this way, no additional components were added directly to the standards or samples, which may cause problems for other pesticides in a standard mixture or sample extract.

A compilation of the obtained recovery and RSD values for HTpSPE clean-up as compared to PSA dSPE clean-up and raw extracts (five replicates at two spiking levels in four matrices) is shown in Fig. 5 (Table 3S, for detailed numerical values).

The results for HTpSPE were exceptional with average recovery for individual pesticides between 90 and 104% and RSD values of 0.3–4.1% ($n = 5$) for a mixture of seven representative pesticides. Summarising, the precision of recovery was generally best using HTpSPE clean-up. For almost all pesticides, recovery obtained after HTpSPE was closer to 100% than recovery by the other methods of comparison. It was noticeable that raw extracts and sample extracts after dSPE with PSA, considered over all pesticides, matrices and spiking levels, resulted in a similar direction of deviation and nearly the same value (either >100% or <100% recovery). This clearly points out that dSPE with PSA is not a sufficient clean-up for providing precise recovery and reflects the impressions obtained both by digital documentation of TLC foils and by the TICs of LC–MS.

Despite the remarkable chemical diversity of the pesticides used in this study, the observed matrix effects (ion enhancement or suppression) did not show any relationship to the pesticides' chemical properties (for a full list of pesticides with corresponding substance classes and specific properties, Table 1S). However, pesticides eluting in time windows with a huge matrix background (Fig. 4) generally gave poor recovery. For both raw extracts and PSA dSPE extracts, clear ion suppression occurred in all matrices for the same pesticides (chlorpyrifos and in most cases for acetamiprid and fenarimol), whereas extracts after HTpSPE showed no matrix effect (Fig. 5). In cucumbers at a spiking level of 0.1 mg/kg, chlorpyrifos gave a recovery of 71.6% in raw extracts, 70.2% in extracts after dSPA with PSA, and 94.8% after HTpSPE. Signal enhancement did not appear as frequently for the methods of comparison, but was detected for azoxystrobin in all matrices at a spiking level of 0.5 mg/kg and was also apparent for pirimicarb to some extent. HTpSPE extracts did not show this matrix enhancement effects in any case.

As plant materials contain a large variety of matrix components such as sugars and peptides as well as plant phenols, organic acids, free fatty acids, chlorophyll or carotenoids in different amounts of composition, the matrix effects of the four matrices examined in this study varied recognisably from each other (Fig. 5). This variation between different matrices is already known and reported [16,17,20,21].

Although the received recovery for sample raw extracts and extracts after PSA dSPE did not show significant differences between the used concentration levels over the different matrices (sometimes enhancement or suppression at the 0.1 mg/kg spiking level and sometimes enhancement or suppression at the 0.5 mg/kg spiking level), RSD values did. At the 0.1 mg/kg level, matrix had a stronger influence on the ionisation of the analyte, and RSD values were higher (Table 3S). This effect has also been reported already [21]. As HTpSPE extracts are nearly free of co-extracted matrix compounds, this influence on RSD values did not occur after HTpSPE, as expected. With RSD values of less than 4%, the average repeatability of each pesticide in each matrix was highly satisfactory.

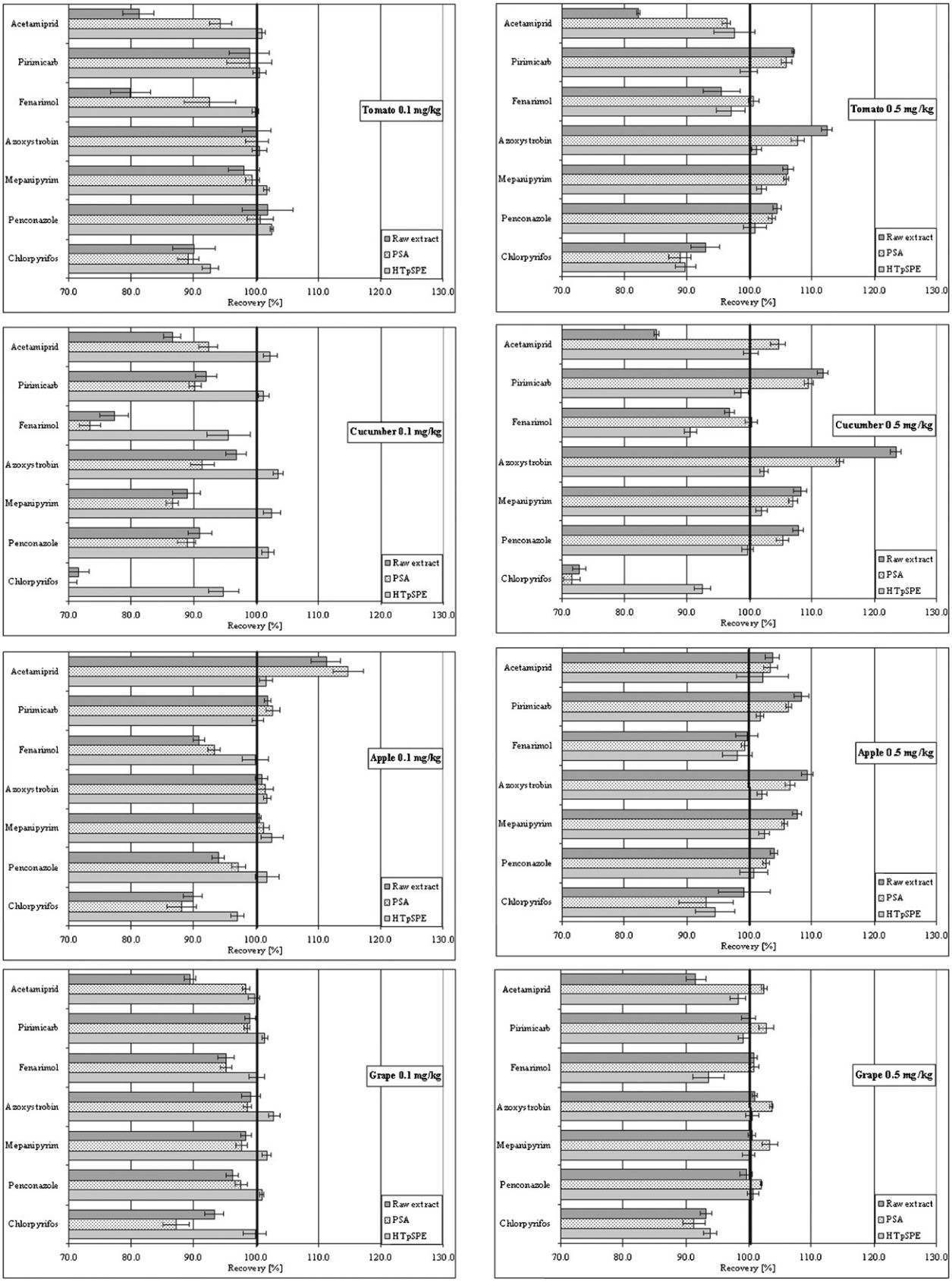

Fig. 5. Recovery results (LC–MS) from QuEChERS raw extracts, after dSPE clean-up with PSA, and after HTpSPE clean-up for seven representative pesticides spiked at 0.1 and 0.5 mg/kg spiking levels in tomatoes, cucumbers, apples and grapes ($n = 5$) (pesticides ordered by increasing retention time).

4. Conclusions

Due to the huge variety of different commodities, which have to be analysed, it is unlikely that perfectly matching blank matrices can be found to satisfactorily compensate for matrix effects with matrix-matched standards in multi-residue methods. Nevertheless, the application of matrix-matched calibration standards is actually the most common way to avoid matrix effects in LC–MS or GC–MS. However, it is more useful to improve sample preparation techniques to provide reliable results of pesticide residues. Planar solid phase extraction (HTpSPE) was shown to be a cost-effective, reliable and rapid alternative to common clean-up techniques such as dispersive and column SPE or gel permeation chromatography. Shifting the clean-up process onto planar thin-layer phases allows a very efficient, high-throughput clean-up requiring only small sample volumes and a solvent consumption of only 1 mL per sample. Additionally, this methodology is nearly fully automated, and due to very clean extract calibration, can simply be performed with pure solvent standards.

The new approach was proven successful with a mixture of pesticides of various substance classes in different fruit and vegetable matrices. Average recovery of seven representative pesticides at two spiking levels from four different matrices was near 100% with relative standard deviations below 4%, confirming the powerful clean-up. Since there were no losses during clean-up and no matrix effects, low recovery over the complete process of residue analysis clearly indicated problems with extractability from the matrix.

As this concept was performed for the first time in this study, this technique is still in its infancy, but has great potential for the improvement and expansion to other fields of trace analysis, such as for polycyclic aromatic hydrocarbons or mycotoxins. However, after having fixed the principles, greatly increasing the number of pesticides – not only LC amenable, but also GC amenable ones – and including problematic matrices like tea or spicery will be the next challenges.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.06.108.

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