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RESEARCH ARTICLE

Applicability of Ultra Performance Convergence Chromatography, a New Generation of Supercritical Fluid Chromatography, for the Analysis of Pesticide Residues

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Abstract

Monitoring and controlling wide variety of pesticide residues is a crucial challenge of food safety. In our study ultra-performance convergent chromatography (UPC²), as the new generation of supercritical fluid chromatography coupled with ESI-MS/MS system was applied to separate a set of pesticides to investigate their chromatographic behavior under various UPC² conditions. 30 components were selected representing the GC and LC measurable components. Capacity factors obtained from LC and GC runs UPC²-PDA were compared. Based on our data UPC² should be considered as an alternative chromatographic approach with separation mechanisms not yet fully characterized. Interestingly the type of mobile phase modifier influences the ionization in an ESI-MS system.

Keywords

ACQUITY UPC², Pesticides, LC-GC, SFC

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1 Introduction

From the middle of the 20th century the increased industrialization of food processing and agriculture made pesticides more frequent in our life. Treating crops with pesticides is believed as a proper tool to maintain stable supply of agricultural products to serve the needs of the increasing world population. However by applying pesticides the number of food safety issues is also raised therefore the registration and the monitoring of these chemicals are inevitable. More than 300 pesticides are registered nowadays. [1]

These days pesticides analysis is preferably carried out by using GC or LC combined with mass spectrometry, predominantly with MS/MS or TOFMS [2, 3]. In some cases detection is based on UV. [4] The selection of separation method depends on the volatility and the thermostability of the target components. If targeted analytes are sufficiently volatile, or can be converted to volatile derivate with chemical modification, without decomposition, GC is the preferred method for separation due to the higher achievable chromatographic resolution. If the analyte contains more polar and/or thermally unstable compounds, the application of LC separation is the appropriate choice [5].

Supercritical fluid chromatography (SFC) as an alternative chromatographic approach might also have feasibility in pesticide analyses. However the application of SFC in this field was highly ignored until these days and only limited literature is available [7]. Probably this is mainly due to hardware-related technical limitations, among which the insufficient reproducibility of former SFC instrumentation was considered the most severe one [6]. Recently appeared SFC hardware solutions from Agilent Technologies called ultraperformance supercritical fluid chromatography (UHSFC) and by Waters Company called ultra-performance convergent chromatography (UPC²) are advertised attributed to be able to achieve fast and easily reproducible results. Additionally since CO_2 (as the main mobile phase) can easily and reproducibly be modified with numerous co-eluents, e.g., hexane, acetonitrile, methanol, ethyl acetate etc., it is claimed that either GC-like and/or LC-like conditions can be similarly set in this system [8].

In this study we challenged convergence chromatographic separation to a carefully selected set of analytes that similarly contained representatives of those compound-types, which are typically considered as GC or LC amenable ones [9]. Our goal was to investigate some basic chromatographic properties (capacity factor and peak shape) of these selected pesticides and comparing the chromatographic results of LC and GC runs related to the same compound.

2 Materials and methods

2.1 Reagents

HPLC-grade acetonitrile (CH_3CN), methanol (CH_3OH) and formic acid (HCOOH) were delivered by VWR; Radnor, PA, USA. CO_2 was obtained from Merck (Darmstadt, Germany). The standards (aldrin, azinphos-methyl, azoxystrobin, boscalid, brompropylate, carbendazim, chlorpropham, diazinon, dichloran, dichlorvos, dimethoate, esfenvalerate, fenhexamid, flutriafol, folpet, heptachlor, pendimethalin, permethrin, piperonyl-butoxide, pirimicarb, pirimiphos-methyl, prochloraz, quintozene, spiroxamine, tau-fluvalinate, tebufenpyrad, thiabendazole, triadimenol, trifloxystrobin, ά-endosulfan.) were obtained from Sigma-Aldrich (Schnelldorf, Germany), Milli-Q-Plus ultra-pure water system from Millipore (Merck-Millipore, Milford, MA, USA) was used throughout the study.

Stock solutions were prepared by dissolving an accurately weighted portion of the pesticides (approximately 10 mg powder or liquid) in 5 ml of an appropriate solvent, and they were stored at -18 °C. Working mixtures (10.0 μg mL^{-1}) were obtained by further dilution with CH_3CN and CH_3OH individually to the UPC2 PDA detection. The selection of appropriate solvents based on the type of the applied co-solvent. Using the single compound working solutions, two 0.6 μg mL^{-1} multi-compound mixtures were prepared. Analytes were split into two mixture solutions to exclude the simultaneous presence of isobaric compounds (e.g., fenhexamid (M+H+ :302.0) and flutriafol(M+H+ :302.1)) in the same mixture. Both mixtures contained also common compounds such as Boscalid and Piperonyl-butoxide. Two working mixtures were prepared twice either in CH_3CN or CH_3OH for different UPC²-MS experiments. In the LC-ESI-MS experiments, mixtures were dissolved in 80:20 v/v% water and the appropriate mobile phase solvent CH_3CN and CH_3OH were used.

2.2 Instrumentation

2.2.1 UPC²-PDA System

The chromatographic measurement was performed using ACQUITY UPC² system (UPC²) (Waters, Milford, MA, USA) with binary solvent manager, sample manager, column manager convergence chromatography manager and PDA (Photodiode Array Detector) detector. The separations were carried out via Acquity UPC² C18 column (3 mm × 100 mm, 1.8 μm), the mobile phase was CO_2 (99.97 % purity) and CH_3OH or CH_3CN were used as co-eluents (CoElu). The mobile phase gradient was the following: 0-1 min: 3%CoElu, 1-9 min: 20%CoElu, 9-9.5: 20 to 3%CoElu, 9.5-13 min: 3% CoElu at a flow rate of 1.5 mL min^{-1}. In the case of CH_3CN the post run was kept until 14.5 min. The injected sample volume was 5 μl. The back pressure of the system was 1500 psi and column temperature was set on 40, 50, 60 and 70 °C. The detection was performed with PDA, between 210-400 nm wavelength.

2.2.2 UPC²-ESI-MS

QTRAP 3200 triple quadrupole-linear ion trap mass spectrometer (Applied Biosystems/Sciex, Foster City, CA, USA) was applied as detection system. The instrument was equipped with a Turbo V interface and Turbo Ion Spray probe (Applied Biosystems), operating in positive ion mode. The UPC²-ESI-MS was coupled with an Agilent 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) UPC² flow rate 1.5 mL min^{-1} and the HPLC flow rate 0.2 mL min^{-1} passed thought the splitter and the mixture was introduced to the ESI. The HPLC pump carried 50:50 v/v% CH_3CN : water with 0.1 v/v% HCOOH. Since the controlling of HPLC-ESI-MS and UPC² systems with one PC was unsolvable, the systems were driven by two separate computers.

The UPC²-ESI-MS coupled system was able to work only with manual injection. The gradient was the same described before. The back pressure of the system was 1500 psi. This value was set after the coupling and applied to the UPC²-PDA method because by MS coupling the back pressure regulator was unable to keep neither 3000 nor 2000 psi pressure previously tried to set. Figure 1 shows the UPC²-ESI-MS system.

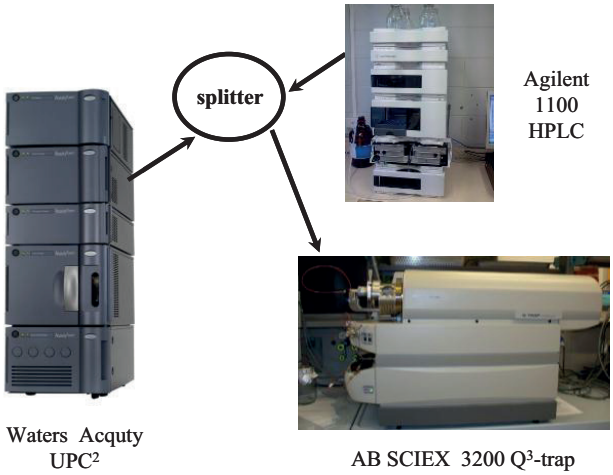

Fig. 1 Applied UPC²-ESI-MS system

2.2.3 HPLC-ESI-MS System

The above described QTRAP 3200 triple quadrupole-linear ion trap mass spectrometer (Applied Biosystems/Sciex; Foster City, CA, USA) was coupled with an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA, USA) by XBridge TM C18 column (3.5 mm × 100 mm × 3.5 μm; Agilent) using

with gradient elution. The eluent consisted of water containing 0.1 v/v% HCOOH (eluent A) and CH_3CN or CH_3OH (eluent B). Gradient elution was set as follows: 0−1 min 5% B; 1-30 min up to 100 % B; 30-35 min 100 % B; followed by 5-min equilibration at 5 % B. The flow-rate was 0.3 ml min^{-1} while the injection volume was 5 µl. The column temperature was set to 25 °C. The optimum settings of the HPLC-ESI-MS/MS coupling were as follows: ion spray voltage: 5500 V; curtain gas (N2): 10 psi; ion source gas: 50 psi; turbo gas: 10 psi; desolation temperature: 450 °C; collision activated dissociation gas: 5.0 arbitrary units. The components were monitored from mixture, the temperature was set to 30, 40, 50, and 60 °C, respectively.

Capacity factor "k" was calculated from the TR with the following formula:

$$k = (T_R - T_D)/T_D$$

Where TR is the retention time, TD is dead time, except of the GC MS data obtained from literature (Waters Application) [10]. The k values were used to compare the behavior of the target compounds under different chromatographic conditions.

3 Results and discussion

The first purpose of our research was to choose the target compounds. The selection was based on former studies [4, 10, 11] and special attention was paid to pick only LC-measurable components, only GC-measureable pesticides and the group that can be determined both way. The selected compounds (depicted in Fig. 2) should have met the following criteria: (i) should be extractable with the citrate buffer of QuEChERS method, ii) should have different pKo/w values and (iii) their retention times should be distributed evenly over the entire chromatographic timescale. In the case of GC-measurable components the selections were made based on the date pool of EURL and the Waters application note.

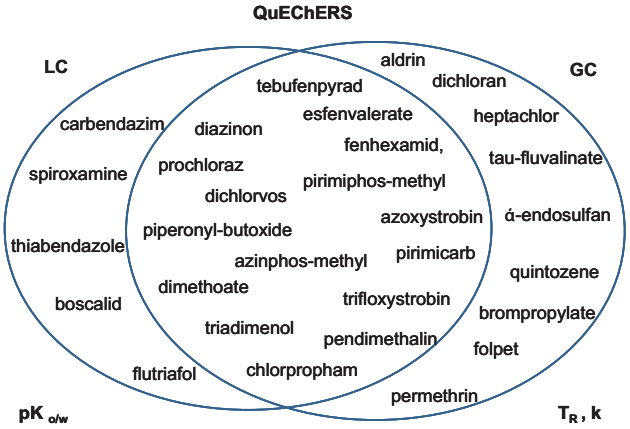

Fig. 2 The 30 choosen compounds

In the first experiment MS method was elaborated. All pesticides were diluted to 1µg mL^{-1} stock solution and injected individually to the QTRAP 3200 triple quadrupole-linear ion trap mass spectrometer by a syringe. The MS parameters were optimized to these compounds.

3.1 Results observed with UPC²-PDA coupled system

Originally the a Waters Acquity UPC² was coupled with a PDA detector that can only detect the target compounds if they are present in huge quantities 10.0 µg mL^{-1}, which has a high detection limit that makes this detector type unable to measure real-life samples that are containing the pesticides in lower amount. Therefore it was necessary to use the different systems that could detect different compounds in lower concentration. The UPC²-PDA system could detect all 30 compounds except spiroxamine, because at the pH of the sample this component did not show any UV activity [12].

Molecules present in the Waters GC application as well as measurable by LC-MS were selected. They were ranked based on their capacity factors (Table 1 and 2) . These rankings were compared among the different methods, and conclusions about the behavior of the pesticides in supercritical conditions were drawn.

The comparison of capacity factors of runs with methanol (LC) and methanol as co-eluent (UPC²) showed that azinphos-methyl, diazinon, prochloraz and triadimenol behaved similarly under GC and UPC² conditions, while chloropham followed the rules of LC chromatography in this new system while the behavior of dichlorvos and piperonil-butoxid showed similarities both that of under LC and GC conditions. X mean no any similarity to between the delaminated sytem retention queue (Table 1).

Table 1 Ranced and compared capacityfactors in the case of CH_3OH

CH_3OH	LC	UPC²	GC	Similarity
Azinphos-methyl	3	9	9	GC
Chlorpropham	5	5	3	LC
Diazinon	8	4	5	GC
Dichlorvos	2	1	2	GC/LC
Dimethoate	1	10	4	x
Permethrin	11	6	10	x
Piperonyl-butoxide	9	7	8	GC/LC
Pirimiphos-methyl	7	2	6	x
Prochloraz	6	11	11	GC
Tau-fluvalinate	10	3	1	x
Triadimenol	4	8	7	GC

The capacity factors of runs with acetonitrile (LC) and acetonitrile as co-eluent (UPC²) (Table 2) were different from those obtained with methanol (Table 1). Distinctively more components showed similar elution behaviour to GC conditions. Apart from dichlorvos and piperonil-butoxide only permethrin behaved as LC measurable. On the contrary the behaviour of

dimethoate piromiphos-methyl, and azinphos-methyl showed no similarity neither GC nor LC-like (Table 2)

Table 2 Ranced and compared capacityfactors in the case of CH_3CN

CH_3CN	LC	UPC²	GC	Similarity
Azinphos-methyl	4	6	9	x
Chlorpropham	6	2	3	GC
Diazinon	7	5	5	GC
Dichlorvos	2	7	2	LC/GC
Dimethoate	1	10	4	x
Permethrin	11	3	11	LC/GC
Piperonyl-butoxide	9	7	8	LC/GC
Pirimiphos-methyl	8	4	6	x
Prochloraz	5	9	10	GC
Tau-fluvalinate	10	1	1	GC
Triadimenol	3	8	7	GC

3.2 Results acquired with UPC²-ESI-MS and HPLCESI-MS coupled systems

Tests with the UPC²-HPLC-ESI-MS system showed that the number of detectable components depended on the quality of the eluent. Out of the 30 pesticides CH_3OH allowed the detection of 25 compounds while using CH_3CN as eluent only 19 pesticides could be detected.

By the LC-MS method all LC-compatible compounds were detected and surprisingly two other, only GC-compatible molecules (namely folpet and alpha- endosulphan). In this method CH_3CN was the stronger eluent, but in the hyphenated UPC² system proved to be less effective because less component were measurable. In the Figure 3 the chromatograms of the LC –MS system is depicted. In the case of using LC-MS with CH_3CN as eluent the peak shapes were Gaussian with less than 0.3 min baseline peak width.

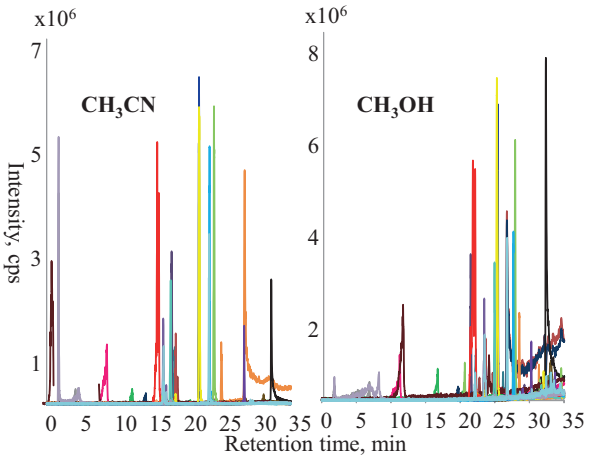

Fig. 3 Elution profiles obtained with the LC-MS system using methanol (left) or acetonitrile (right) as eluent in 40 °C

Figure 4 shows the comparison of UPC²-ESI-MS elution profile gained by the use of CH_3CN and CH_3OH. It is clearly perceptible that by using CH_3OH containing eluent more components shoved regular peak shape. However it must be mentioned the baseline with this system is not so straight. The reason of this phenomenon is the assistant solvent from the HPLC pump, which was necessary for the ionization of the compounds. Firstly We coupled the UPC² directly to the ESIMS system but in this case there was no ionization observed. Therefore assistant pump was used to the coupling. (Fig. 1) However the assistant solvent increased the ionization of the compunds efficiently, it also elevated baseline.

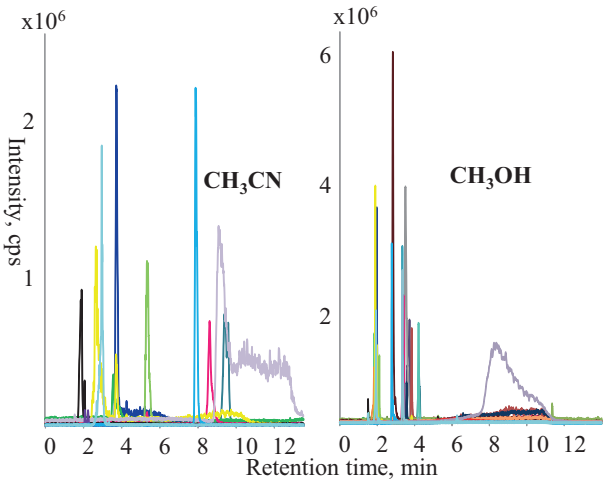

Fig. 4 Elution profiles from the UPC2-ESI-MS system using methanol(left) or acetonitrile (right) as eluent in 40 °C

To handle this problem the UPC² system was coupled successfully either with single quadruple or with ESI TOF MS. The application of these stable coupled systems could result a scientific breakthrough in the field of pesticide analysis.

In general by increasing the temperature the density of the CO_2 eluent decreases and so does its solvating ability [13, 14]. Therefore, the higher the temperature the later the components would elute from the column. On the other hand no increase was found in the elution times of dimethoate, tebufenpyrade, prochloraz, piperonyl-butoxide with the addition of CH_3CN to the eluent. It suggest in the Fig. 5.

4 Conclusion

All standards examined can be detected with either PDA or MS methods therefore UPC² has a potential new applicability in the field of pesticide analytics. The method needs further optimization before it can be applied in routine laboratories. The separation kinetics of the UPC² system does not resemble that either LC or GC therefore it has to be handled as a new, different method. GC is defined by using a gas as its mobile phase and LC is defined by using liquids as its mobile phase, however UPC² is using both gas and liquid. This convergence

of mobile phases in combination with a far greater choice of stationary phases makes UPC² a powerful additional choice for laboratory scientists.

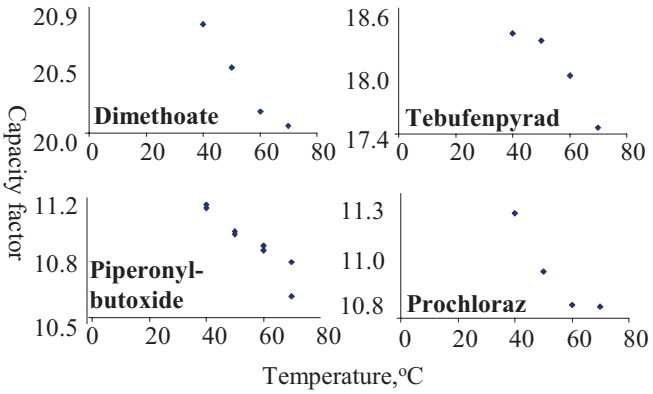

Fig. 5 Elution behaviour in the case of using ancetonitrile coelunent by the examinated UPC² system

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