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Technical Report

# Dimethyl carbonate as a green organic modifier in supercritical fluid extraction of pesticide residue analysis in apple samples

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## Abstract:

In line with the Green Analytical Chemistry principles, the drive to replace conventional organic solvents with more sustainable alternatives is rapidly evolving. This study introduces dimethyl carbonate (DMC) as a novel co-solvent in supercritical fluid extraction (SFE) for the multi-residue analysis of pesticides in apple samples. The extraction performance of DMC was systematically compared with that of acetonitrile (ACN) and bio-ethanol (EtOH) under optimized conditions (30% co-solvent, 150 bar, 70 °C, 1 mL $min^{-1}$ flow rate, and 21 min of total extraction time). A panel of 64 pesticides, covering different chemical classes (e.g., organochlorine, organophosphorus, pyrethroid, carbamate, benzamides, triazoles), polarities (with log $k_{ow}$ ranging from –0.9 to 7.7), and molecular weights (ranging from 141 to 532), were evaluated. The method achieved an average extraction yield of 85% with DMC, while also providing significantly cleaner extracts by reducing the co-extraction of matrix components. These results underline the potential of DMC to enhance the sustainability and efficiency of pesticide residue analysis, closely aligning with the objectives of green analytical practices.

**Keywords: Supercritical fluid extraction, Pesticides, Apple, Green solvents, Dimethyl carbonate**

## 1. Introduction

According to the Green Analytical Chemistry (GAC) principles[1], a highly promising strategy to minimize the side effects of analytical methods involves replacing potentially toxic solvents and/or obtained by non-renewable sources with more environmentally friendly alternatives.

In this frame, dimethyl carbonate (DMC) is an environmentally friendly, non-corrosive, safe handling, and biodegradable chemical, extensively used in several application fields, such as pharmaceuticals, coatings, and lithium-ion battery electrolytes [2]. Additionally, instead of the historical use of toxic and corrosive phosgene as a carbonate source, simple and direct synthesis routes from carbon dioxide ($CO_2$) have been proposed [3]. Thus, also falling within the "carbon capture and utilization" processes, ensuring the reduction of atmospheric $CO_2$ concentration and its conversion into useful chemical products. Firstly introduced in the second edition of the GlaxoSmithKline's solvent selection guide, DMC has been included among the "few issues" solvents, along with water [4]. Thereafter, it has attracted widespread attention as a valuable chemical and/or solvent, and substantial research efforts have been made in the scientific community. Its potential as a green alternative to conventional solvents in both reversed-phase liquid chromatography [5–7] and supercritical fluid chromatography [8] has been explored. The extraction capabilities of DMC toward different target molecules have been also investigated. In 2022, Alfieri et al. optimized an ultrasound-assisted extraction of two lignans from *Linum* species [9]. The extraction performances of a panel of green solvents and two natural deep eutectic solvents (in combination with water in a solvent/water ratio of 80:20) were tested versus methanol and chloroform (used for comparison with published studies). The results showed that, in terms of purity and recovery, DMC was the optimal media for the 6-methoxypodophyllotoxin extraction. DMC has been also employed in an in-syringe extraction coupled to solid phase extraction to assess nicotine in environmental water and sportspersons' urine samples [10]. Extracts enriched in oleanolic acid were obtained by solid-liquid extraction of grape pomace using DMC compared to other tested solvents (ethyl acetate, acetone, n-butanol) [11]. In addition to the highest obtained recovery value, the lowest co-extraction of other matrix components was also achieved.

Supercritical fluid extraction (SFE) represents a reliable alternative to conventional solvent-based extraction methods. Several advantages are provided when $CO_2$ is employed as supercritical fluid due to its non-toxicity, non-flammability, abundancy, renewability (as a by-product of industrial processes, as fermentation or combustion), recyclability, and cost-effectiveness. Supercritical $CO_2$ has a polarity similar to that of *n*-hexane; thus, the addition of a co-solvent is necessary for the extraction of polar molecules [12, 13].

This work aims to expand the use of DMC that, to the best of the author's knowledge, is here firstly proposed as an organic modifier in SFE. The extraction efficiency and selectivity of DMC toward conventional solvents, such as acetonitrile (ACN) and bio-ethanol (EtOH) obtained from edible renewable feedstocks, has been evaluated for multi-residue analysis of pesticides (64 pesticides, representing diverse chemical classes, polarities, and molecular weights) in apple samples. Considering the sustainable and non-toxic nature of $CO_2$ and DMC, the use of DMC in SFE represents a win-win combination. Thus, improving the overall sustainability and automation of the extraction process closely align with the GAC principles. Furthermore, in this research, always following the GAC principles, a low-pressure gas chromatography-tandem mass spectrometry (LP-GC-QqQMS) method was employed, allowing a greater sample loadability and a higher optimal linear velocity allowed by the vacuum conditions of the system, effectively reducing the analysis time at about 10 minutes [14, 15].

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## 2. Materials and methods

### 2-1. Chemicals

The pesticide standards, ACN (grade ≥ 99.9%), EtOH (grade ≥ 99.5%), and DMC (grade ≥ 99%) were purchased from Merck KGaA (Darmstadt, Germany). For each standard compound, a stock solution (10,000 mg $L^{-1}$) was prepared in ACN. A multi-analyte working solution (10 mg $L^{-1}$) was prepared and stored at 4 °C until use. For the construction of six-point calibration curves, mixed standard solutions with different concentrations (5–250 µg $L^{-1}$) were used.

### 2-2. Samples and sample preparation

Six apple samples were purchased in markets located in Messina (Italy). A representative part of each sample was ground in a blender and the obtained apple puree was spiked at a concentration of 15 mg $kg^{-1}$. The mixture was kept for 3 h under magnetic agitation to ensure pesticide incorporation and solvent evaporation. Subsequently, considering the apples' high water content, an appropriate amount of sample was mixed with the Miyazaki Hydro-Protect dehydrating agent (Shimadzu) at a ratio of 4:1 (sample:dehydrating agent). The 0.2 mL stainless steel extraction vessels were completely filled with 170 mg of the obtained mixture (136 mg of apple) and placed into the SFE rack changer.

### 2-3. Instrumentation

### Supercritical fluid extraction system

The Nexera-UC off-line SFE pre-treatment system (Shimadzu, Japan) employed was equipped with: a CBM-40 controller, an SFE-30A auto-extractor connected with a 48-vessel rack changer (equipped with 0.2 mL extraction vessels), an LC-30ADSF $CO_2$ pump, an LC-40ADXR dualplunger parallel-flow pump, an LC-40D make-up pump, an SFC-30A back pressure regulator, a DGU-40 degasser, and an FRC-40 fraction collector. A sequence of static and dynamic extraction was employed. A schematic representation of the employed SFE system is shown in Figure 1.

For each tested solvent, the optimized SFE method is reported in Table 1.

At the end of the dynamic step, 1 min of extraction with 100% of $CO_2$ was employed to transfer the remaining solvent contained in the extraction vessel. Furthermore, in order to avoid analytes carry over, the system was washed for 3 min using 100% of modifier at a flow rate of 1 mL $min^{-1}$.

Immediately after the BPR, a make-up flow was added to the extract to avoid analyte precipitation into the transfer flow line. During the extraction optimization, the make-up flow was tuned to achieve a total solvent flow of 1 mL $min^{-1}$ considering the modifier amount.

A gas-liquid separator allowed the elimination of the decompressed $CO_2$, while the liquid fraction was directly dripped and collected into a 32 mL glass vial without dispersing. The analysis was controlled using Shimadzu LabSolutions version 5.127 software. Each sample was extracted in triplicate (n = 3). To compare and determine the extraction yields for the target compounds, all the extracts were diluted in volumetric flasks of 10 mL or 20 mL to have a controlled dilution volume and directly analyzed by LP-GC-QqQMS.

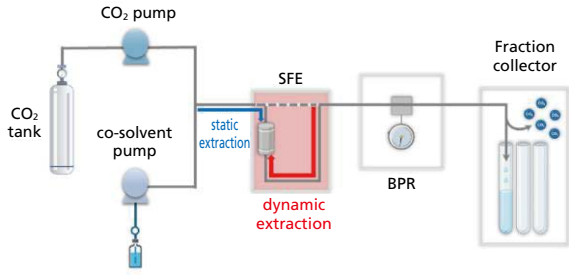


Figure 1: Schematic representation of the employed SFE system

Table 1: SFE extraction conditions optimized for each solvent

| Modifier | Modifier Amount (%) | Flow (mL $min^{-1}$) | BPR (bar) | Extraction T. (°C) | Extraction Time(min) |
|---|---|---|---|---|---|
| ACN | 30 | 1 | 150 | 70 | 10+10 static and dynamic |
| EtOH | 30 | 1 | 150 | 70 | 1+20 static and dynamic |
| DMC | 30 | 1 | 150 | 70 | 1+20 static and dynamic |

### Low-pressure gas chromatography triple quadrupole mass spectrometry

The LP-GC-QqQMS analyses were performed using a GC-2010 Plus system (Shimadzu Corporation, Kyoto, Japan) coupled with a triple quadrupole mass spectrometer (TQ8040, Shimadzu). Injection was performed using an AOC-20i autosampler and a split/splitless injector (injection temperature 280 °C) equipped with a focus liner (volume: 810 µL). The injection was performed in splitless mode (3 µL) using the high-pressure injection mode function (290 kPa for 0.5 min).

To avoid that sub-ambient pressure conditions reach the injector, an uncoated column (0.48 m × 0.1 mm ID) was installed before the separation column, a non-polar Equity-5 [poly(5% diphenyl/95% dimethyl siloxane)] of the following dimension: 5 m × 0.53 mm ID × 0.53 µm *df*. Both the columns were provided by Merck KGaA. The oven was temperature programmed as follows: 40 °C (2 min) to 320 °C raised at 30 °C $min^{-1}$. Helium was used as carrier gas with an average constant linear velocity of 100 cm $s^{-1}$ (into the separation column).

Electron ionization was performed at 70 eV. The interface and ion source temperatures were 320 and 280 °C, respectively. The collision gas was argon at a pressure of 200 kPa. The targeted analyses were carried out in the multiple reaction monitoring (MRM) acquisition mode (acquisition frequency: 3.3 Hz). To ensure univocal identification, two different MRM transitions (quantifier and qualifier) were monitored for each compound (Table S1). The SCAN analysis was performed with an acquisition frequency of 3.3 Hz and a mass range of 50–600 *m/z*. The GCMS Solution v.4.45 software (Shimadzu) was used for data collection and processing.

## 3. Results and discussion

The extraction capability of DMC as an organic modifier in SFE was here firstly evaluated versus conventional solvents (ACN and bio-EtOH). A panel of 64 pesticides (62 out of those reported in the EU pesticide database), covering different chemical classes (e.g., organochlorine, organophosphorus, pyrethroid, carbamate, benzamides, triazoles), polarities (with *log kow* ranging from –0.9 to 7.7), and molecular weights (ranging from 141 to 532), was selected (Table 2).

The LP-GC-QqQMS chromatogram, acquired in the MRM mode, of an apple sample spiked at a concentration of 15 mg $kg^{-1}$ is shown in Figure 2.

During the method development process, it was decided to use a high pesticide concentration compared to the maximum residue levels (MRLs) established by the European Community [16]. This decision took into account the dilution factor introduced during the extraction step. In fact, the concentration of the extract is approximately 0.1 mg $kg^{-1}$. This approach was specifically chosen to eliminate the need for a concentration step. It is important to underline that, although it is not the research aim, the developed methods. have also been tested at a concentration of 0.05 mg $kg^{-1}$ (considered as a fair compromise between the MRLs of the analyzed pesticides in the range 0.01–15 mg $kg^{-1}$) to evaluate their feasibility respecting the European MRLs.

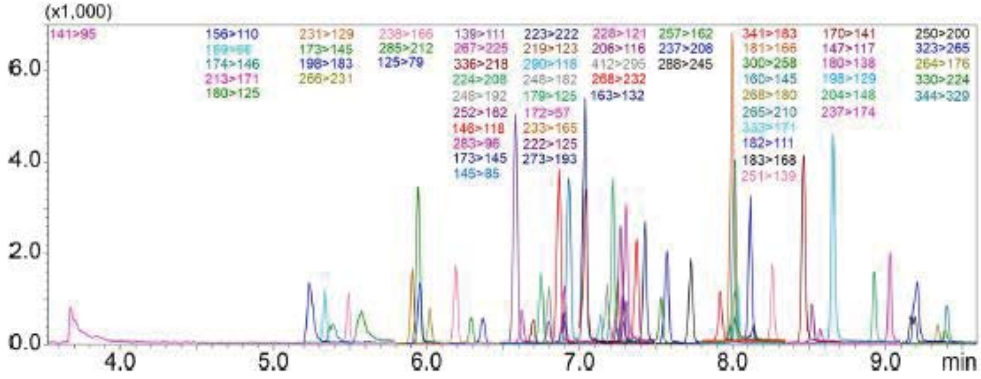


Figure 2: LP-GC-QqQMS chromatogram, acquired in the MRM mode, of an apple sample spiked at 15 mg $kg^{-1}$ 1 concentration level

Table 2: List of the analyzed compounds along with molecular weight (MW), *log kow*, recovery, and matrix effect values (measured at the best extraction conditions of each solvent)

| Compound | MW | log *kow* | ACN | | EtOH | | DMC | |
|---|---|---|---|---|---|---|---|---|
| | | | Rec% | %ME | Rec% | %ME | Rec% | %ME |
| Methamidophos | 141.1 | -0.9 | 98.1 | 66.2 | 96.8 | 117.7 | 94.1 | 111.7 |
| Chlorpropham | 213.7 | 3.5 | 91.8 | 69.3 | 108.6 | 106.7 | 83.5 | 117.3 |
| Omethoate | 213.2 | -0.9 | 100.2 | 159.7 | 112.0 | 305.3 | 103.6 | 331.8 |
| Pencycuron | 328.8 | 4.8 | 96.8 | 50.0 | 110.3 | 32.3 | 99.0 | 52.8 |
| Clothianidin | 249.7 | 1.3 | 89.9 | 141.9 | 104.7 | 57.3 | 88.6 | 316.0 |
| Flonicamid | 229.2 | 0.8 | 108.2 | 69.9 | 99.2 | 176.5 | 64.4 | 262.6 |
| Diphenylamine | 169.2 | 3.5 | 79.6 | 69.5 | 94.9 | 102.5 | 68.1 | 119.3 |
| Terbufos | 288.4 | 4.5 | 85.7 | 46.8 | 93.5 | 74.4 | 60.2 | 82.6 |
| Pyrimethanil | 199.3 | 2.9 | 97.2 | 75.2 | 106.8 | 119.6 | 95.1 | 114.5 |
| Propyzamide | 256.1 | 3.2 | 89.9 | 52.7 | 109.3 | 75.0 | 71.9 | 86.3 |
| Chlorothalonil | 265.9 | 2.9 | 101.8 | 436.5 | 109.9 | 382.4 | 74.1 | 435.9 |
| Pirimicarb | 238.3 | 1.7 | 97.3 | 65.6 | 112.3 | 96.7 | 94.2 | 106.2 |
| Dimethoate | 229.3 | 0.8 | 78.8 | 63.9 | 90.0 | 91.2 | 66.4 | 94.1 |
| Vinclozolin | 286.1 | 3.1 | 96.7 | 59.1 | 112.2 | 85.5 | 53.3 | 139.2 |
| Fenpropidin | 273.5 | 5.5 | 99.3 | 65.4 | 113.2 | 79.4 | 109.5 | 69.9 |
| Fenchlorphos | 321.5 | 5.1 | 96.0 | 52.0 | 91.2 | 77.7 | 59.5 | 89.7 |
| Diethofencarb | 267.3 | 2.8 | 89.7 | 69.6 | 106.8 | 111.7 | 91.8 | 99.5 |
| Tetraconazole | 532.0 | 4.4 | 98.3 | 74.0 | 112.8 | 116.9 | 104.7 | 98.7 |
| Dichlorobenzophenone | 251.1 | 4.4 | 89.6 | 63.9 | 101.2 | 90.7 | 78.4 | 86.5 |
| Cyprodinil | 225.3 | 3.1 | 98.4 | 77.8 | 104.8 | 114.8 | 89.5 | 107.8 |
| Procymidone | 284.1 | 3.0 | 102.8 | 57.9 | 110.6 | 88.1 | 85.9 | 92.2 |
| Methidathion | 302.3 | 2.4 | 87.2 | 91.3 | 112.1 | 125.7 | 90.5 | 118.0 |
| Fluopyram | 396.8 | 4.5 | 101.2 | 70.0 | 100.5 | 102.4 | 111.1 | 87.0 |
| Penconazole | 284.2 | 4.4 | 95.6 | 74.4 | 112.5 | 109.7 | 106.7 | 98.7 |
| Pendimethalin | 281.3 | 5.2 | 80.8 | 58.2 | 102.6 | 104.6 | 84.1 | 86.3 |
| Quinalphos | 298.3 | 4.4 | 95.5 | 76.4 | 108.2 | 111.1 | 84.4 | 115.8 |
| Ancymidol | 256.3 | 1.6 | 100.9 | 84.5 | 113.6 | 121.2 | 105.4 | 118.5 |
| Bupirimate | 316.4 | 2.7 | 84.0 | 64.9 | 109.2 | 90.0 | 74.5 | 120.3 |
| Cyflufenamid | 412.4 | 5.0 | 87.9 | 66.2 | 110.8 | 78.5 | 107.2 | 84.2 |
| Cyproconazole | 291.8 | 2.9 | 91.1 | 91.3 | 109.5 | 134.5 | 88.0 | 125.9 |
| Diniconazole | 326.2 | 4.2 | 102.7 | 70.9 | 108.3 | 115.4 | 84.8 | 114.1 |

| Compound | MW | log *kow* | ACN | | EtOH | | DMC | |
|---|---|---|---|---|---|---|---|---|
| | | | Rec% | %ME | Rec% | %ME | Rec% | %ME |
| Flusilazole | 315.4 | 3.7 | 106.1 | 75.8 | 108.6 | 139.8 | 99.2 | 115.2 |
| Flutriafol | 301.0 | 2.3 | 102.0 | 75.4 | 115.7 | 135.8 | 108.5 | 100.6 |
| Mepanipyrim | 223.3 | 3.3 | 84.5 | 71.4 | 116.9 | 109.1 | 107.4 | 96.5 |
| Fludioxonil | 248.2 | 2.6 | 109.0 | 97.8 | 109.3 | 147.9 | 72.5 | 170.3 |
| Kresoxim-methyl | 313.3 | 4.1 | 91.4 | 77.3 | 114.0 | 117.6 | 66.5 | 164.1 |
| Myclobutanil | 288.8 | 2.9 | 110.6 | 70.8 | 113.1 | 119.2 | 64.9 | 156.8 |
| Buprofezin | 305.4 | 3.8 | 92.2 | 73.1 | 96.3 | 105.8 | 75.1 | 113.8 |
| Isoprothiolane | 290.4 | 3.3 | 99.3 | 64.8 | 107.9 | 117.3 | 98.2 | 89.2 |
| Oxadixyl | 278.3 | 1.8 | 101.5 | 100.3 | 113.2 | 140.3 | 102.8 | 128.1 |
| Proquinazid | 372.2 | 3.7 | 110.9 | 52.1 | 109.0 | 80.5 | 71.2 | 83.7 |
| Quinoxyfen | 308.1 | 5.1 | 95.7 | 81.8 | 111.8 | 113.1 | 87.4 | 107.7 |
| Triazophos | 313.3 | 3.5 | 91.7 | 86.2 | 112.9 | 144.0 | 91.7 | 126.1 |
| Fenarimol | 331.2 | 3.6 | 106.3 | 78.4 | 107.2 | 120.7 | 78.1 | 117.7 |
| Bifenazate | 300.4 | 4.2 | 78.1 | 80.3 | 102.0 | 93.9 | 59.2 | 197.6 |
| Fenamidone | 311.4 | 4.1 | 87.8 | 87.3 | 113.4 | 111.1 | 83.4 | 116.2 |
| Fenazaquin | 306.4 | 5.7 | 85.2 | 75.5 | 95.8 | 114.7 | 61.6 | 116.7 |
| Tebufenpyrad | 333.9 | 4.5 | 94.6 | 60.9 | 104.8 | 95.0 | 77.8 | 100.3 |
| Phenothrin | 350.5 | 6.2 | 87.0 | 72.7 | 100.1 | 99.6 | 81.7 | 98.2 |
| Phosalone | 367.8 | 4.4 | 93.2 | 89.5 | 112.6 | 162.9 | 82.5 | 149.1 |
| Bromopropylate | 428.1 | 4.8 | 95.6 | 48.4 | 93.7 | 79.2 | 61.7 | 80.2 |
| Bifenthrin | 422.9 | 6.0 | 81.5 | 79.7 | 95.4 | 105.9 | 66.7 | 106.0 |
| Fenpropathrin | 349.4 | 5.7 | 83.8 | 72.9 | 102.8 | 107.3 | 72.8 | 106.3 |
| Prochloraz | 376.7 | 4.6 | 89.7 | 108.9 | 108.8 | 172.0 | 109.1 | 137.7 |
| Bitertanol | 337.4 | 4.2 | 104.3 | 105.9 | 103.3 | 198.5 | 100.7 | 142.2 |
| Fenbuconazole | 336.8 | 3.2 | 109.7 | 124.5 | 108.0 | 230.5 | 108.4 | 155.6 |
| Pyridaben | 364.9 | 5.2 | 93.7 | 78.7 | 102.8 | 115.5 | 45.3 | 163.3 |
| Pyridalyl | 491.1 | 7.1 | 98.4 | 77.1 | 94.5 | 118.7 | 67.1 | 108.1 |
| Benzovindiflupyr | 398.2 | 4.0 | 101.6 | 99.3 | 114.9 | 177.3 | 87.4 | 142.8 |
| Tau-Fluvalinate | 502.9 | 7.7 | 72.9 | 197.9 | 88.9 | 457.4 | 57.9 | 439.4 |
| Difenoconazole | 406.3 | 4.0 | 105.6 | 126.4 | 108.2 | 232.7 | 107.0 | 133.8 |
| Famoxadone | 374.4 | 5.0 | 97.5 | 249.1 | 108.3 | 361.2 | 109.5 | 181.3 |
| Indoxacarb | 527.8 | 4.8 | 102.3 | 158.7 | 114.2 | 290.1 | 108.4 | 182.6 |
| Azoxystrobin | 403.4 | 3.7 | 106.7 | 158.0 | 110.3 | 453.2 | 96.1 | 281.3 |

## 3-1. SFE optimization

In order to achieve the highest recovery, during the SFE optimization the following parameters were evaluated: co-solvent type and amount, extraction temperature, static and dynamic extraction time. Considering that at a lower flow rate the extraction efficiency increases because of an extended contact between the analytes and the supercritical fluid [17], a flow of 1 mL $min^{-1}$ was employed and a pressure of 150 bar was applied. All the tests were performed in triplicate, varying one experimental parameter at a time. For each solvent, six extraction methods were tested (Table 3).

First of all, considering the low polarity of the neat $CO_2$ for the extraction of pesticides with such a wide range of polarity, the co-solvent amount was tested across the10–50% range. As expected, the use of 30% of modifier allowed higher average recovery compared to 10%. However, at 50%, yields dropped as a result of a considerable change of the $CO_2$ critical parameters after the addition of a large amount of modifier [12].

In addition to the modifier amount, also the temperature showed a great influence on the extraction performances with the best recoveries obtained at a temperature of 70 °C. The increased temperature improves extraction efficiency and yield by enhancing the solubility and diffusion of compounds. This is because higher temperatures increase the kinetic energy of molecules, leading to faster diffusion and greater solubility. However, excessively high temperatures can lead to degradation of some compounds, so an optimal temperature range should be determined [18].

Using 70 °C , a percentage increase of the overall recovery of 46%, 29%, and 30% for ACN, EtOH, and DMC, respectively, was observed respect to 30 °C . Increasing the dynamic extraction time from 10 to 20 min, the pesticide yields slightly increased using EtOH (average 12%) and DMC (average 10%). No improvements were observed with ACN. Finally, an increase in the static extraction time from 1 to 10 min was also tested, leading to slightly better results only for extraction with ACN as co-solvent.

To compensate for possible matrix effects, the recovery values were calculated by comparing the analytes area values of a spiked blank sample with those of a post-spiked extract. The yields obtained employing the best extraction conditions of each solvent are reported in Table 2 and Figure 3 (along with the error bars).

Table 3: SFE variables tested for the method optimization of ACN, EtOH, and DMC, respectively

| | Met_1 | Met_2 | Met_3 | Met_4 | Met_5 | Met_6 |
|---|---|---|---|---|---|---|
| **ACN** | | | | | | |
| Static ext. | 1 min 10% | 1 min 30% | 1 min 30% | 1 min 30% | 1 min 50% | 10 min 30% |
| Dynamic ext. | 10 min 10% | 10 min 30% | 20 min 30% | 10 min 30% | 10 min 50% | 10 min 30% |
| Temperature | 30 °C | 30 °C | 30 °C | 70 °C | 70 °C | 70 °C |
| **EtOH** | | | | | | |
| Static ext. | 1 min 10% | 1 min 30% | 1 min 30% | 1 min 30% | 1 min 50% | 10 min 30% |
| Dynamic ext. | 10 min 10% | 10 min 30% | 20 min 30% | 20 min 30% | 20 min 50% | 20 min 30% |
| Temperature | 30 °C | 30 °C | 30 °C | 70 °C | 70 °C | 70 °C |
| **DMC** | | | | | | |
| Static ext. | 1 min 10% | 1 min 30% | 1 min 30% | 1 min 30% | 1 min 50% | 10 min 30% |
| Dynamic ext. | 10 min 10% | 10 min 30% | 20 min 30% | 20 min 30% | 20 min 50% | 20 min 30% |
| Temperature | 30 °C | 30 °C | 30 °C | 70 °C | 70 °C | 70 °C |

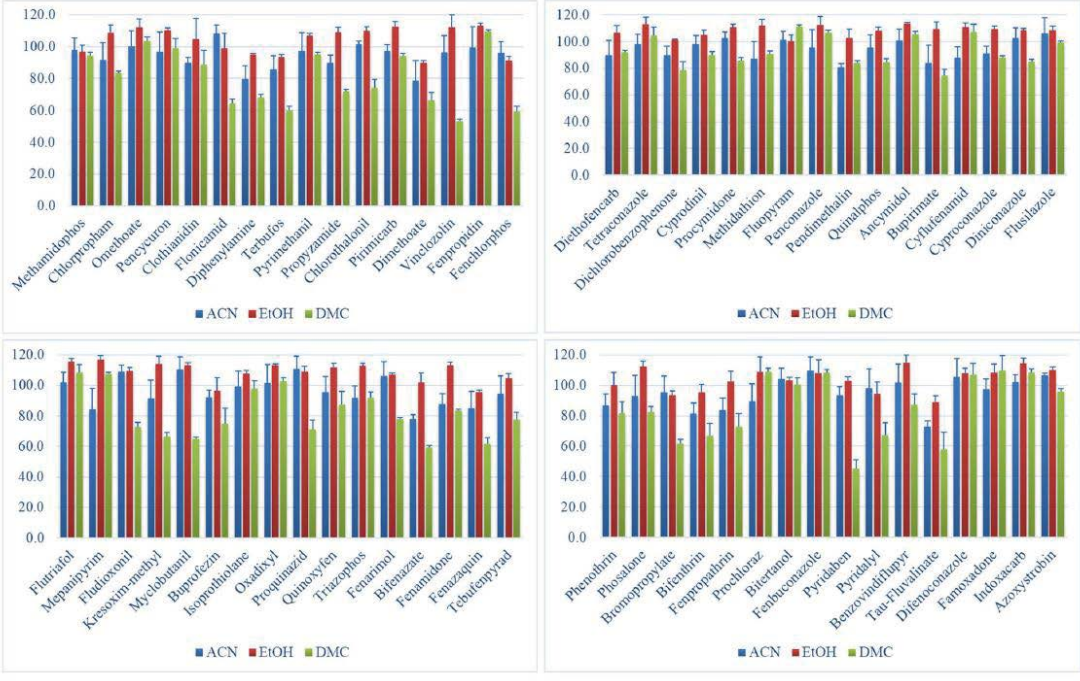


Figure 3: Bar graphs showing pesticide recoveries obtained using the best extraction method for each solvent

Considering ACN as co-solvent, the measured recovery values ranged between 72.9% for tau-fluvalinate and 110.9% for roquinazid, with an average value of 95.1%. Using EtOH, the measured recovery values were in the 88.9–116.9% range (for tau-fluvalinate and mepanipyrim, respectively) with an average value of 106.4%. Finally, with DMC, the recovery values ranged from 45.3% (pyridaben) to 111.1% (fluopyram), with an average value of 85.0%. Although for 15 compounds (namely flonicamid, diphenylamine, terbufos, dimethoate, vinclozolin, fenchlorphos, kresoxim-methyl, myclobutanil, bifenazate,fenazaquin, bromopropylate, bifenthrin, pyridaben, pyridalyl, and tau-fluvalinate), recoveries below 70% were obtained using DMC, the SANTE/11312/2021 guidelines [19] allow for values outside the 70–120% range if they exhibit satisfactory precision. In this case, all these compounds showed an RSD ≤ 12%. Except for flonicamid and dimethoate (which have a *log* $k_{ow}$ of 0.8 and are better extracted with more polar co-solvents), no correlation was observed between the low recoveries and the *log* $k_{ow}$ values of these pesticides. Even with highly lipophilic pesticides, such as tau-fluvalinate and pyridalyl (*log* $k_{ow}$ > 7), higher recoveries were obtained with EtOH, probably due to its higher ability to form hydrogen bonds [11]. Additionally, these low recoveries could be related to a poor pesticide solubility in DMC, although no supporting literature data are currently available.

## 3-2. Matrix effect and evaluation of solvents selectivity

For an in-depth investigation of the extraction capabilities of the employed co-solvents, the matrix effect and the amount of co-extracted matrices were measured. Matrix effect (%) values were calculated as the ratio between the analytes' peak area in a blank matrix spiked after extraction and the analytes' peak area in pure solvent solution. All the calculated %ME values were reported in Table 2. %ME values equal to 100% correspond to the absence of matrix effect. Values below or above 100% correspond to signal suppression and enhancement, respectively [20].

Using ACN as co-extraction solvent, the %ME values (average 90.5%) ranged from 46.8% (terbufos) to 436.5% (chlorothalonil), with 52 pesticides characterized by a negative value, and 12 by a positive one. Using EtOH, the %ME values (average 140.1%) ranged from 32.3% (pencycuron) to 457.4% (tau-fluvalinate), with 18 pesticides characterized by a negative value, and 46 by a positive one. Finally, DMC provides %ME values in the 52.8–439.4% range (again for pencycuron and tau-fluvalinate, respectively), with 19 pesticides characterized by a negative value, and 45 by a positive one (average 137.4%).

The amount of co-extracted matrices was measured at the best extraction conditions for each solvent. Specifically, an amount of 52 mg, 28 mg, and 5 mg was extracted with EtOH, ACN, and DMC, respectively. Therefore, an amount about 10 times lower was obtained with DMC compared to EtOH.

As previously reported, the higher selectivity observed with DMC is related to its lower ability to form hydrogen bonds with other abundant apple analytes (as carbohydrates) which were extracted in high yield with other solvents able to form such bonds, as EtOH [11].

Considering the GC injection volume (3 µL), when DMC is employed as co-solvent, the amount of co-injected matrix components was circa 0.75 µg per analysis (compared to 7.8 µg with EtOH). Thus, preserving the GC and the MS systems and reducing their maintenance. Moreover, it should be emphasized that, in order to fulfill the EU MRLs, a concentration step needs to be performed, further increasing the amount of co-injected matrix. The difference in co-extracted matrices amount is also clearly visible in Figure 4, showing a comparison of the SCAN chromatograms acquired for the EtOH, ACN, and DMC apple extracts.

## 3-3. Method evaluation and analysis of commercial apple samples

As previously reported, the optimized extraction method using DCM has been further evaluated by spiking a blank apple sample at a concentration level of 0.05 mg $kg^{-1}$. The final extract has been concentrated under a gentle stream of nitrogen and injected into the LP-GC-QqQMS system. As reported in Table S1, an average S/N value of 199 was obtained, ranging from 17 for Cyflufenamid (MRL 0.06 mg $kg^{-1}$) to 882 for Methidathion (MRL 0.03 mg $kg^{-1}$). Only two pesticides, namely Methamidophos and Pencycuron, were under the limit of detection.

Six apple samples, collected from local markets (Messina, Italy), were analyzed using the developed method using DMC as co-solvent for SFE. Three replicates were performed for each sample. All the pesticides determined were at a concentration lower than the 0.05 mg $kg^{-1}$ limit, respectively, 17 in sample one, 1 in sample two, 3 in sample three, 3 in sample four, and 2 in sample five.

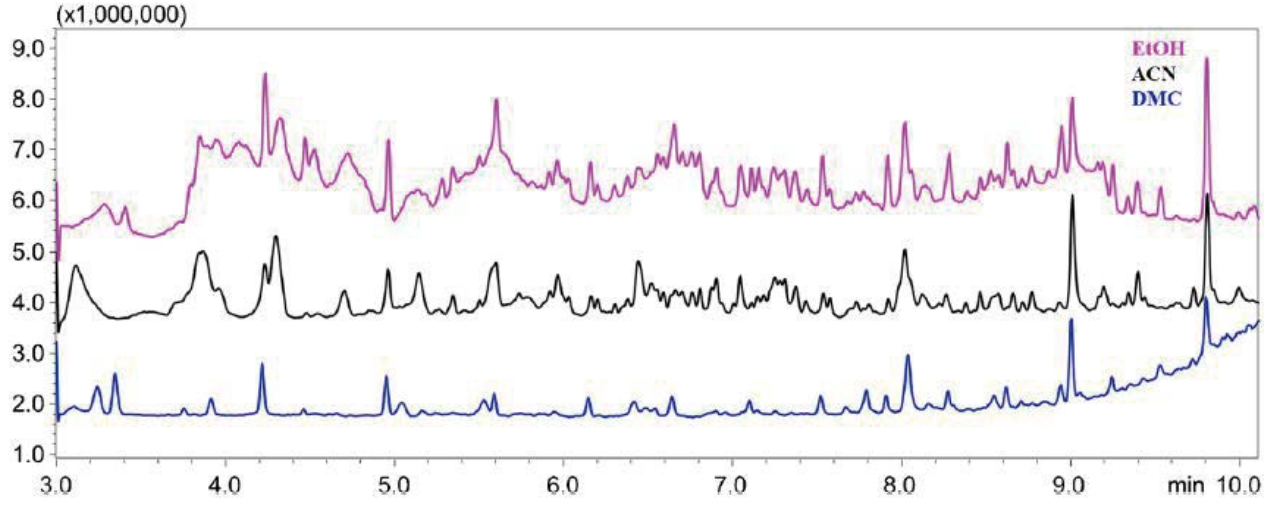


Figure 4: Comparison of LP-GC QqQMS chromatograms (acquired in SCAN mode) of the EtOH, ACN, and DMC apple extracts (not concentrated)

Table 4: Comparative information between the proposed SFE method with other published methods

| Sample preparation | Separation/ detection system | Solvent amount | Sample amount | No. of analytes | Sample preparation time (min.) | Recovery range | LoQ (μg $L^{-1}$) | Ref. |
|---|---|---|---|---|---|---|---|---|
| dLLμE | MD-GC/MS | Carbon tetrachloride (100 μL) Acetone (400 μL) | 5 g | 24 | 8 | 60–105% | 0.30–7.30 | [21] |
| Modified QuEChERS | UPLC-MS/MS | Acetonitrile (20 mL) | 10 g | 14 | 19 | 70.1–118.2% | 0.10–1.0 | [22] |
| Modified QuEChERS | UHPLC-MS/MS | Acetonitrile (20 mL) | 10 g | 22 | 31 | 74.4–118.1% | 2.0–10.0 | [23] |
| Modified QuEChERS | HPLC-MS/MS | Acetonitrile (10 mL) | 10 g | 120 | 17 | 70.0–120.0% | 0.01 | [24] |
| SFE | GC-MS/MS | Dimethyl carbonate (23 mL) | 0.136 g | 64 | 25 | 45.3–111.1% | ≤ MRL (except for methamidophos and pencycuron) | This study |

*dLLμE*: dispersive liquid-liquid microextraction, *MD-GC/MS*: multidimensional gas chromatography-mass spectrometry

## 3-4. Comparison with other studies and greenness evaluation

A comparison with previously published papers, focused on pesticide determination in apple, is herein made (Table 4) in terms of sample preparation type and time, employed amount of solvents and sample, along with partial figure of merit, namely recovery percentage and limit of quantification.

In terms of solvent amount, the dLLμE [21] was characterized by the lowest amount of employed solvents in addition to the lowest sample preparation time (8 min). Three different protocols based on a modified QuEChERS were characterized by the consumption of a high amount of acetonitrile (10 or 20 mL) and by an amount of sample of 10 g in all the cases [22–24]. The approach proposed in this study required the lowest sample amount, namely 136 mg. Despite the high consumption of DMC (23 mL considering extraction and system wash), its non-toxic, biodegradable, and renewable properties enhance the method compatibility with the GAC principles.

In this framework, the AGREEprep analytical metric tool was employed to evaluate the method greenness [25]. Using DMC, three green points, related to principles 2 (hazardous materials), 5 (size economy of the sample), and 10 (operator's safety), were obtained with an overall score of 0.45. On the contrary, the method had the lowest score in principles 1 (ex situ analysis), 4 (mass of generated waste), and 8 (energy consumption), related to the long extraction time (20 min) and to the use of a sophisticated SFE apparatus. Using bio-EtOH, considering one additional hazard pictogram compared to DMC, a slightly lower score (0.42) was obtained. As expected, the lowest score (0.22) was obtained for the SFE procedure using ACN as co-solvent, thus considering the use of 13 mL of hazardous materials in addition to the non-sustainability and/or renewability of such a solvent.

The major green character of the herein proposed extraction using DMC versus the EN 15662:2018 QuEChERS official procedure for pesticide extraction [26], with a score of 0.26, is shown in the easy-to-read pictograms in Figure 5.

## 4. Conclusions

Under the optimized extraction conditions, the use of DMC as a co-solvent in SFE demonstrated promising performance in the analysis of pesticide residues in apple samples.

The method achieved an average extraction yield of 85% across the 64 analyzed pesticides, spanning a broad range of chemical classes, polarities, and molecular weights. Notably, DMC provided cleaner extracts with significantly lower amounts of co-extracted matrix components compared to conventional solvents like ACN and EtOH. Despite the recovery for certain analytes being below 70%, the results remained within acceptable precision limits according to SANTE guidelines. Overall, DMC not only fulfills the analytical requirements for pesticide residue determination but also offers a greener, more sustainable alternative that supports enhanced instrument maintenance and aligns with the principles of GAC.

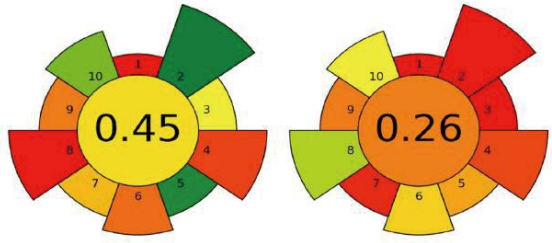


Figure 5: AGREEprep comparison between SFE with DMC (left) and QuEChERS official method reported in the EN 15662:2018 (right) extractions

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