Novel multi-residue method for rapid determination of pesticides in wines using GC-MS/MS

Ultra-sensitive, simple, and cost-effective analysis by Bruker μDROP and the EVOQ GC-Triple Quadrupole system

Abstract

An innovative multi-residue method was developed to identify and quantitate a diverse panel of pesticides customarily used during the growth of wine grapes in a single analysis. Pesticides were extracted from wine using the Bruker μDROP method, which was previously used in the successful extraction and quantitation of pesticides in water samples [1]. Within the EVOQ GC-MS/MS detection/quantitation workflow, this rapid, reliable, and simple sample enrichment methodology easily meets current analytical validation criteria according to SANTE/12682/2019 guidelines for pesticide residue analysis in foods [2]. Method validation has been conducted in many different types of commercial wines. The inherent high-sensitivity of the method supports ~100-fold dilution with water prior to extraction, such that a negligible matrix effect was observed among different wine samples. A method detection limit (MDL) of 0.5 ng/mL (ppb) has been established for all pesticides, and in all wine types, analyzed within this study.

Keywords: Wine, pesticides, μDROP, EVOQ GC-TQ system, vineyard management, regulatory compliance

Authors: Diego Martín, Javier López, and Miguel Ángel Pérez; Bruker Applications Development Laboratory, Madrid, Spain

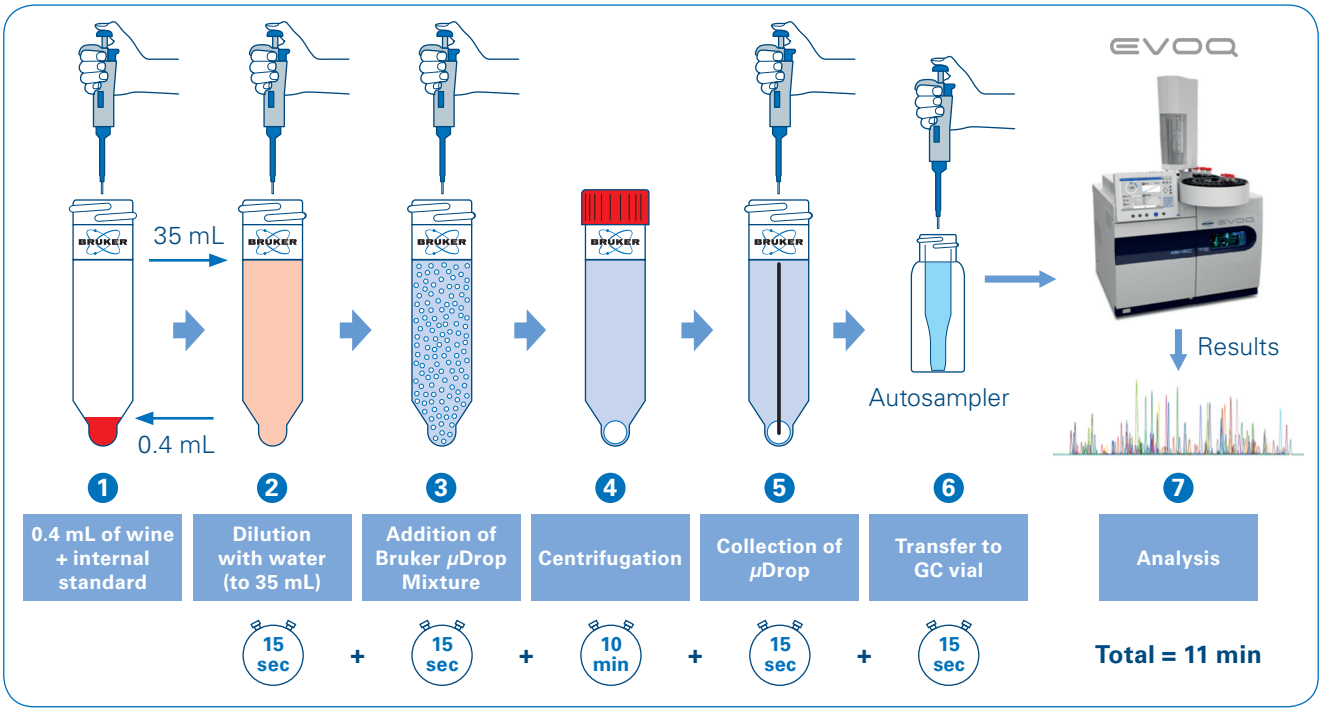


Figure 1. Bruker µDROP sample preparation workflow for wine analysis. Beginning with 0.4 mL of wine, samples are enriched using Bruker µDROP and ready for automated GC-MS analysis in 11 minutes.

Introduction

Over its long history, the cultural and economic importance of viticulture has both benefited from and led to considerable evolution, from changes in farming practices to sophisticated analytical techniques to monitor flavor profiles and assure product safety. The majority of wines today are produced from cultivated varieties of *Vitis vinifera* grapes, and, as is the case for many commercial crops, protection against grape damage made by harmful organisms or disease is a continual concern. Powdery or downy mildew and black rot, for example, can lead to the complete destruction of a grape crop, and various fungicides, among other pesticides, are common "modern" production tools. Such plant protection products (PPPs) are subject to strict regulations within the EU (as in other wine-producing regions) regarding authorized and appropriate use to support sustainability and safety [3-5]. This environmental protection extends to vineyard workers and wine consumers as well. Worldwide wine consumption has recently been estimated to be 244 million hectoliters per year [6], and guaranteeing product safety for such high volumes is a critical task.

As plant protection products, pesticides may be used in grape production (growth), storage, and transport. The potential physico-chemical transfer of regulated PPPs to the grape components (e.g., skin, juice, and seeds) during vinification varies with the type of wine to be

Table 2. Analytical conditions

Parameters	Conditions
GC	**Bruker 436 GC**
Injector	1079 inert, PTV injector
Column	BR-5ms, 30 m x 0.25 mm, 0.25 micron
Total run time	29 min
Column flow	He, 1 mL/min
Autosampler	Bruker 8400
MS Detector	**Bruker EVOQ Triple Quadrupole**
Source	EI
Source temperature	280°C
Transfer line temperature	280°C
MS operation mode	MRM
Collision gas	Ar, 2 mTorr
Detector	EDR
Software	Bruker Compass TQ

Table 1. Pesticides analyzed, in order of GC retention time (RT). The internal standard, triphenyl phosphate, is shown in italics.

N°	Pesticides	RT [min]	N°	Pesticides	RT [min]
1	**Diazinon**	10.04	33	**Benalaxyl**	14.66
2	**Propyzamide**	10.04	34	**Trifloxystrobin**	14.72
3	**Pyrimethanil**	10.12	35	**Quinoxyfen**	14.89
4	**Fenbuconazole**	10.18	36	**Fluopicolide**	14.96
5	**Chlorpyrifos methyl**	10.91	37	**Fenhexamid**	15.01
6	**Parathion methyl**	10.96	38	**Tebuconazole**	15.17
7	**Vinclozolin**	10.96	39	**Propargite**	15.22
8	**Metalaxyl**	11.08	40	***TPP (Internal Standard)***	*15.28*
9	**Fenitrothion**	11.44	41	**Proquinazid**	15.30
10	**Diethofencarb**	11.69	42	**Zoxamide**	15.48
11	**Chlorpyrifos ethyl**	11.72	43	**Iprodione**	15.65
12	**Triadimefon**	11.85	44	**Bifentrin**	15.79
13	**Tetraconazole**	11.87	45	**Bromopropilate**	15.83
14	**Cyprodinil**	12.28	46	**Fenamidone**	16.02
15	**Chlorfenvinphos**	12.42	47	**Tebufenpyrad**	16.09
16	**3,5-Dichloroaniline**	12.43	48	**Tetradifon**	16.38
17	**Penconazole**	12.43	49	**Benthiavalicarb-isopropyl**	16.54
18	**Quinalphos**	12.60	50	**Fenarimol**	17.02
19	**Triadimenol**	12.67	51	**Famoxadone**	17.12
20	**Procymidone**	12.68	52	**Metrophenone**	17.12
21	**Fenoxycarb**	13.06	53	**Pyraclostrobin**	17.45
22	**Mepanipyrim**	13.13	54	**Permethrin**	17.62
23	**Endosulfan (alpha)**	13.17	55	**Pyridaben**	17.75
24	**Fludioxonil**	13.28	56	**Cyfluthrin**	18.28
25	**Hexaconazole**	13.32	57	**Cypermethrin**	18.55
26	**Iprovalicarb**	13.50	58	**Fenvalerate**	19.31
27	**Flusilazole**	13.56	59	**Esfenvalerate**	19.50
28	**Kresoxim-methyl**	13.57	60	**Difenoconazole**	19.80
29	**Myclobutanil**	13.58	61	**Indoxacarb**	19.86
30	**Cyflufenamid**	13.78	62	**Deltamethrin**	20.05
31	**Oxadixyl**	14.20	63	**Azoxystrobin**	20.43
32	**Endosulfan (beta)**	14.22	64	**Dimethomorph**	20.64

produced. Likewise, the types of PPPs used on grapes in the production of wine can vary significantly with the types of pests to control. Highly lipophilic pesticides (log K_{ow}>5) present little risk of passing from grapes into wine. Most of the pesticides used in agriculture, however, have log K_{ow} values between 2.0 and 4.8.

The maximum residue levels (MRLs) of pesticides established by the European Commission in effort to protect consumer safety differ according to their risk [7]. The majority of MRLs fall between 10-500 µg/kg (ppb), although some are much higher (e.g., vinclozolin and zoxamide, MRL 5,000 ppb). For pesticides without an established MRL, the minimum value of 10 ppb is taken. MRLs for processed products such as wine are subject to a transformation factor with respect to the MRLs of their raw material, i.e., the wine grape. According to current regulations [8], a transformation factor of 1 is taken for wine, such that the MRLs for the wine are equal to those of the grape.

The utility of GC-MS/MS for the detection of pesticides has been well established, but sample preparation or enrichment is generally required to detect residual or trace levels of these compounds. Pesticide screening workflows for wine and other foodstuffs based on classic techniques, such as liquid/liquid extraction, often require considerable hands-on laboratory time and relatively high volumes of organic solvents per sample, requiring appropriate hazardous material disposal. The more recent introduction of other commonly used methods, including Solid Phase Extraction (SPE) and dispersive solid-phase extraction (dSPE)-QuEChERS offer reproducibility and sensitivity, but with necessary costs of specific columns and kits in addition to laboratory time.

Bruker µDROP is a proprietary, miniaturized, and ultra-sensitive method for the analysis of aqueous samples, including wine. A detailed description of the technique and its successful application to water sample analysis have been previously described [1]. This technique involves three liquid phases: a water-immiscible solvent (extractant), a water-miscible solvent (dispersant), and the aqueous sample. In this technique, a mist of fine microdroplets of the extractant is dispersed into the aqueous phase, resulting in an immediate extraction of analytes. Via centrifugation, the fine microdroplets subsequently form a single microdroplet containing all extracted analytes. This simultaneous extraction, with high target recovery (70-120%) and enrichment (up to

1000 times), can include compounds of diverse chemical structures. All pesticides amenable to GC-MS may be determined in a single injection following the μDROP preparation. Further, the method is rapid, low-cost, and environmentally friendly, compliant with current green chemistry recommendations.

This application note describes the use of the Bruker μDrop methodology for the determination of 63 pesticides in five distinct types of wine via GC-MS/MS. Young red wine, crianza (aged) red wine, white wine, sparkling Cava (white) wine, and sweet Port wine were analyzed. The advantages of sensitivity, speed, and ease of use, along with low sample and solvent consumption, provide a powerful and cost-effective means of sample enrichment for the identification and quantitation of trace levels of pesticides in wine samples.

Materials and Methods

Wine samples

Young red wine, crianza (aged) red wine, white wine, sparkling Cava (white) wine, and sweet Port wine were purchased from commercial sources.

Pesticides standards

Wine samples were spiked with a mix of 63 pesticides (Table 1) commonly used within European vineyards at concentrations as indicated within the text. Pesticide standards were obtained from AccuStandard, Inc. (New Haven, CT, USA). Triphenyl phosphate (TPP) was used as the internal standard (IS).

Sample preparation using Bruker μDROP and GC-MS/MS analysis

The sample preparation scheme is outlined in Figure 1. In brief, 0.4 mL of each wine (blank or spiked, including the internal standard) was introduced into a 50 mL Bruker centrifuge tube (p/n: 1850435). Each sample was diluted to 35 mL with DI water, followed by the addition of the Bruker μDROP Solvent Extraction Mixture #1 from the (ready-to-use) μDROP kit (p/n: 1845184). Following centrifugation, the μDROP droplet was retrieved using a manual pipet and introduced into an autoloader for automated analysis by the EVOQ GC-TQ system. Details of the GC-MS/MS analysis conditions are outlined in Table 2. Bruker Compass TQ software (Bruker Daltonics) was used for EVOQ GC-TQ data screening and quantitation. Data evaluation rules were set to meet the validation criteria according to SANTE/12682/2019 guidelines for pesticide residue analysis in foods [2], such that any reported values higher than the setpoint would trigger a red flag to indicate that the compound requires review. An example MRM chromatogram is shown in Figure 2.

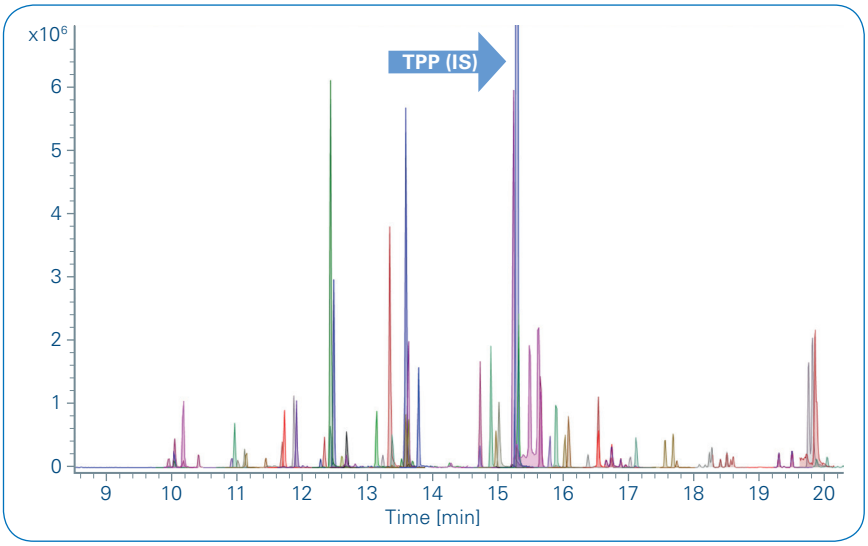


Figure 2. Overlaid MRM chromatogram of a young red wine spiked with 63 common vineyard pesticides at 0.5 ppb following Bruker μDROP sample enrichment.

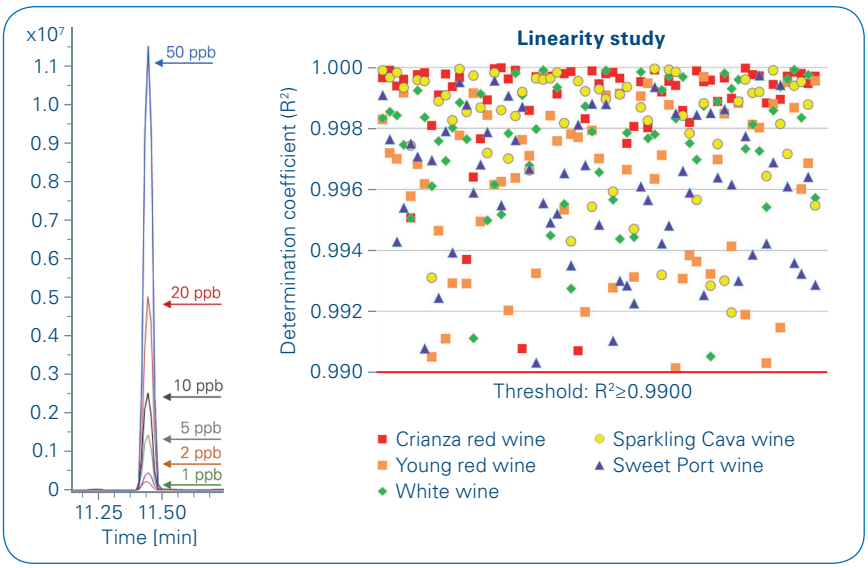


Figure 3. Fenitrothion calibration in extracted sparkling Cava wine (left). Summary of R^2 values for the six-point calibration curves of each pesticide, in each wine matrix (right).

Evaluation of method linearity, precision, specificity, and sensitivity

Detection linearity was evaluated with spiked wine samples prepared at 1, 2, 5, 10, 20, and 50 ppb. Each prepared sample for each wine type included all 63 target pesticides. Samples were extracted using the Bruker *μ*DROP method as described above. In order to evaluate the precision and specificity of the method, five sample extractions were made in each of the spiked wines at the 10 ppb calibration level. As modern TQ MS systems operating in MRM mode produce noise values close to zero for many transitions, method sensitivity was experimentally determined according to the parameters of Method Reporting Limit (MRL) and Method Detection Limit (MDL) rather than by calculations based on signal to noise values.

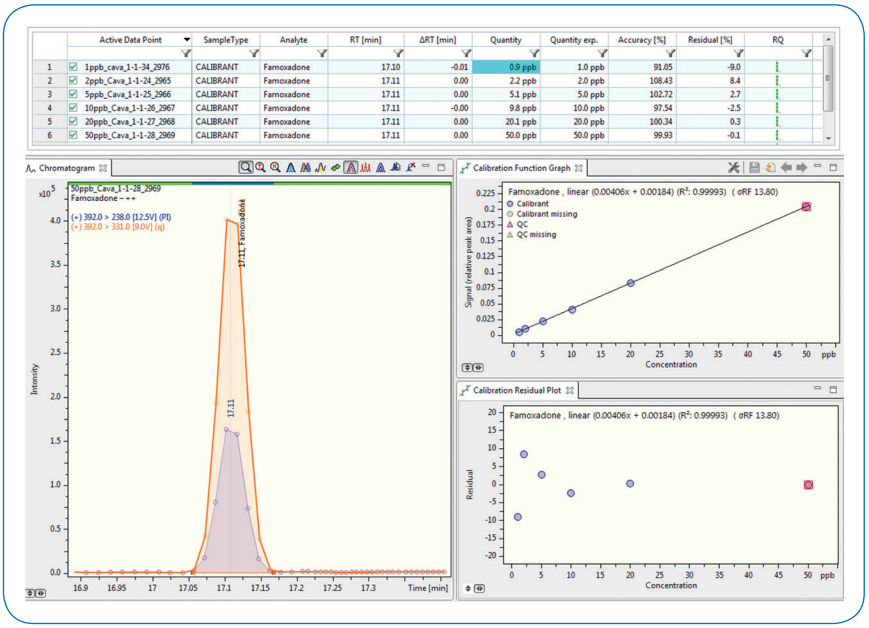


Figure 4. Calibration curve for famoxadone in sparkling Cava wine as generated within the Compass TQ software, indicating the dispersion of each point of the calibration curve in both graphic and table forms.

Results

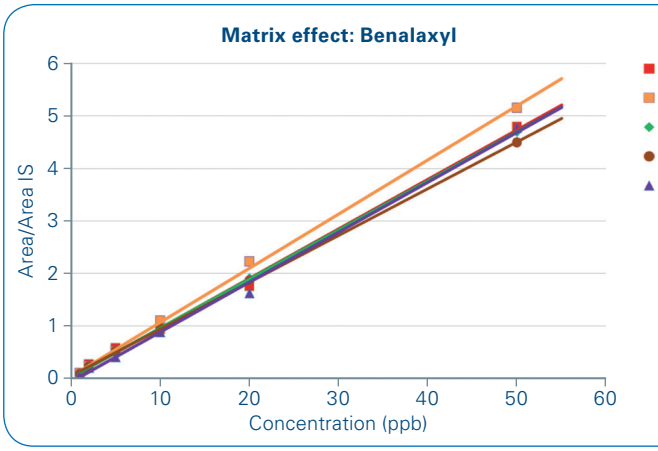
Linearity

The EVOQ GC-TQ analysis method following Bruker *μ*DROP extraction had excellent linearity for all pesticides in all wine matrices. The target criteria of R^2 ≥0.990 was fulfilled for all compounds, with most having R^2 values greater than or equal to 0.996 (Figure 3). The RSD (%) for the calibration curves for all pesticides were 5-25%. A dashboard view of the calibration curve of famoxadone in sparkling Cava wine is shown in Figure 4.

Matrix effect

The effect of the signal of each compound in the different types of wine has been studied by comparing the slope of the corresponding calibration curves. Figure 5 compares the calibration curves of benalaxyl in all of the wines tested. As shown, a difference is observed, with a coefficient of variation (RSD) of 5.3% between the slopes of the calibration curves.

In the tested method, the initial dilution of wine samples approximately 1:100 with water minimized matrix effects for all wine types examined. This facilitates routine operation regardless of the type of wine to be analyzed. Further, a calibration curve prepared from any wine sample could be used to quantify any other wine sample.



Wine	Slope	Slope ratio	Matrix effect vs. Cava (%)
Cava	0.0892	1.000	0.00%
White	0.0936	0.953	4.70%
Young red	0.1032	0.864	13.57%
Crianza red	0.0944	0.945	5.51%
Port	0.0953	0.936	6.40%
	RSD=5.3%		

Figure 5. Matrix effect evaluation via calibration curve comparisons for the pesticide Benalaxyl in all tested wine types.

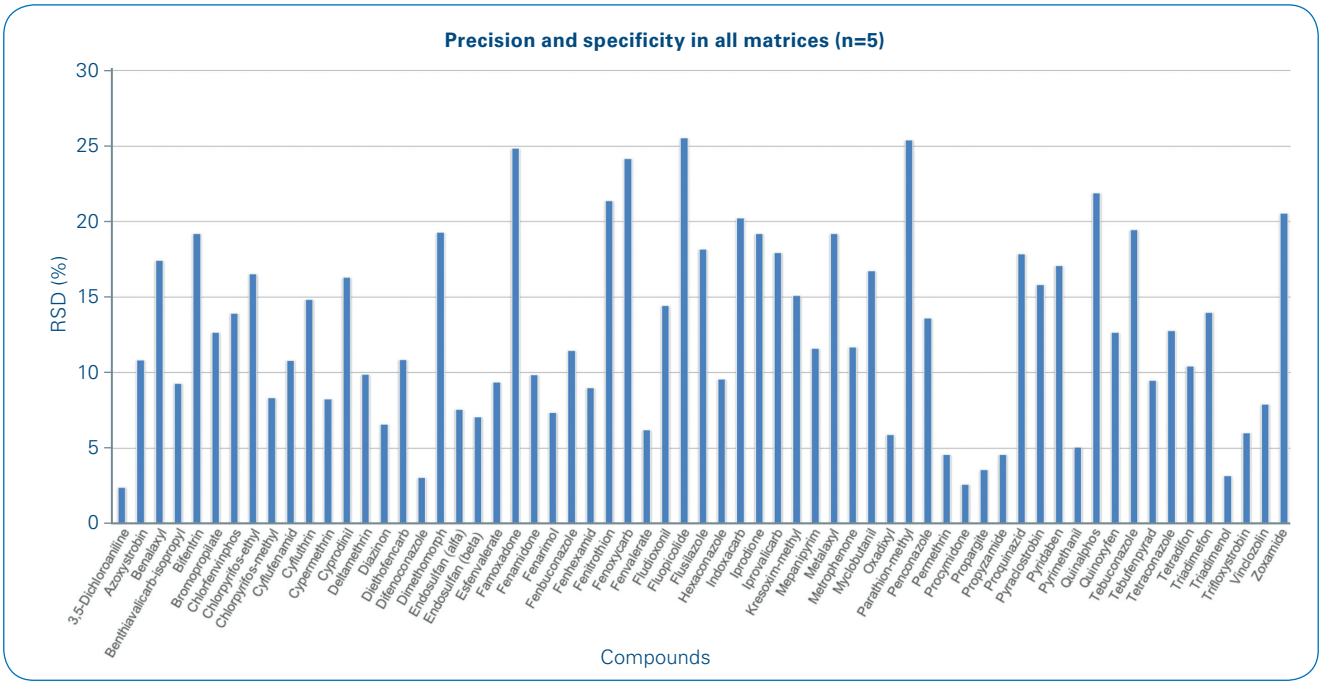


Figure 6. Precision and specificity as evaluated by multiple Bruker µDrop extractions (n=5) of all the compounds at 10 ppb in each of the five types of wine. RSD values were <25% for all pesticides in all wine matrices.

Precision and specificity

As tested, method precision and specificity were excellent. In all wine matrices, at 10 ppb the RSD was <25% for all compounds tested (Figure 6).

Method sensitivity

As previously mentioned, modern TQ MS methods based on MRM transitions have noise near to zero, resulting in S/N values far from analytical reality. Thus, the Method Reporting Limit (MRL) and Method Detection Limit (MDL) are often used as sensitivity descriptors. The Method Reporting Limit (MRL) is defined as the lowest concentration that can be quantified reliably, fulfilling the criteria of precision and accuracy established for the method. In this study, the MRL values correspond to the first calibration point, 1 ppb (e.g., Figure 7A).

The Method Detection Limit (MDL) is defined as the minimum concentration of a target compound detected, considering both the sample preparation and the specific parameters of the method, with both the quantitation ion and the confirmation ion detected with S/N values >10. An MDL <0.5 ppb has been established in this study, as quantitation and confirmation ions could be confirmed in all wine matrices spiked with 0.5 ppb of pesticides (e.g., Figure 7B) following Bruker *µ*Drop extraction and EVOQ GC-TQ analysis.

Discussion

In this study, Bruker *µ*Drop extraction prior to EVOQ GC-TQ analysis has been shown to be a precise, reliable, and sensitive methodology for the simultaneous detection and quantitation of residual pesticides in diverse wine matrices. The current analytical regulatory criteria for 63 common vineyard management pesticides used within the EU were easily met with this workflow.

In addition to high analytical performance, the Bruker *µ*Drop extraction method is straightforward and simple. Requiring very little sample (0.4 mL) and little time, this workflow offers significant cost and time savings over more traditional methods for analysis (Table 3). Multi-residue analyses may be quickly and easily made at every stage of wine production – from wash water testing to pre-fermentation juices to the finished bottle – with minimal product loss.

The unsurpassed enrichment power of Bruker *µ*Drop, together with the high accuracy and speed of analyses using the EVOQ GC-TQ system, is extremely well suited to meet the laboratory necessities of method throughput and sensitivity for rapid determination of target pesticides in wines, as in many other aqueous samples. Further, this methodology significantly reduces the volume of hazardous waste necessary, supporting modern green chemistry principles.

Table 3. Comparison between Bruker µDROP and "traditional methods."

	Bruker µDROP	Liquid/ Liquid Extraction	QuEChERS	SPE
Number of samples to be analyzed	40	40	40	40
Sample volume (wine)	0.4 mL	20 mL	10 mL	10 mL
Estimated time to prepare all samples (*)	11 min	800 min	200 min	60 min
Solvent volume to prepare all samples	120 mL	3200 mL	440 mL	680 mL
Consumables to prepare all samples	None	None	Extraction kit dSPE tubes	SPE cartridges
Laboratory equipment in addition to GCMS	Centrifuge	Glassware Agitator Evaporator	Centrifuge Vortex	Vacuum manifold Evaporator
Sensitivity	♦♦♦♦♦	♦	♦♦	♦♦♦

* ***µDROP***: *Processing 40 samples simultaneously using a centrifuge accommodating 40 tubes*
L/L Extraction: *Processing 6 samples simultaneously using an agitator accommodating 6 funnels*
QuEChERS: *Time estimated by Restek Corporation*
SPE: *Processing 24 samples simultaneously using a vacuum manifold accommodating 24 SPE cartridges*

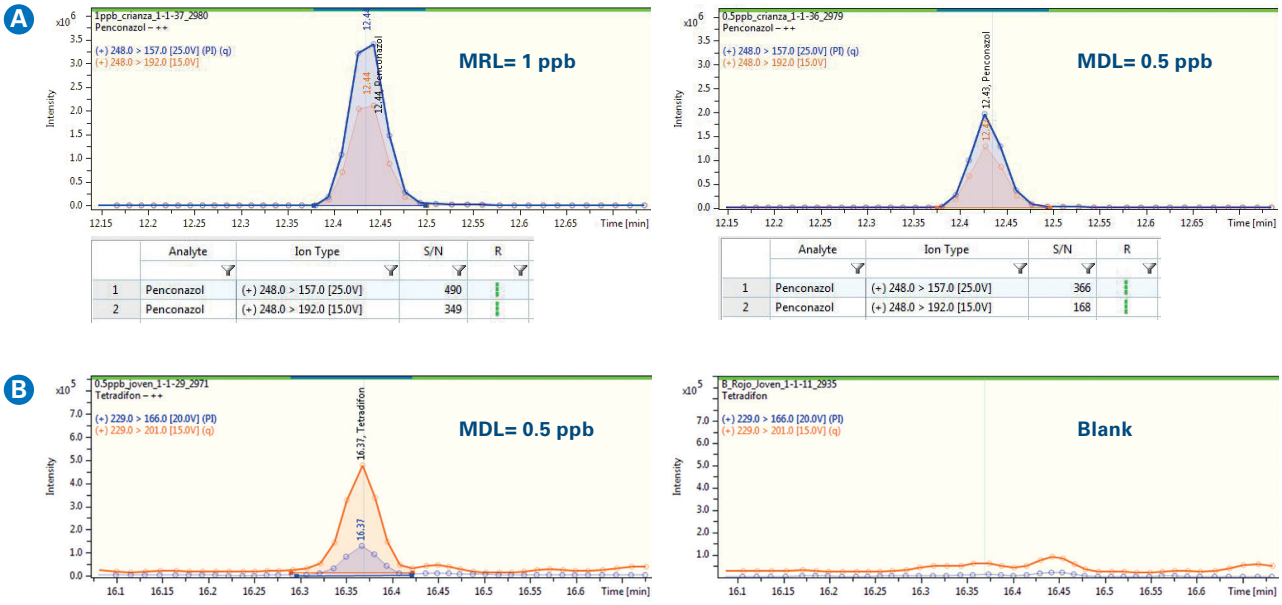


Figure 7. Determination of method sensitivity (as Method Reporting Limits and Method Detection Limits) for Penconazole (A) and Tetradifon (B). The MDL for Penconazol was confirmed with two transitions of S/N>100. For Tetradifon, the MDL of 0.5 ppb (left panel) was apparent relative to the blank (right panel).

Conclusion

- The combination of the Bruker µDROP sample preparation workflow and EVOQ GC-TQ screening offers unique analytical power with excellent linearity, precision, and accuracy to meet the validation criteria of SANTE/12682/2019 guidelines for determination of pesticide residues in foods.
- The method is ultra-sensitive, with sub-ppb detection limits from low sample volumes (0.4 mL). This high sensitivity resulting from its innovative aqueous extraction approach permits a high method dilution factor (~100-fold), broadening the method applicability as matrix effects are negligible for all types of wine.
- The method is extremely cost- and time-effective regarding sample and consumable needs, laboratory personnel, and waste disposal. These features further contribute to its high analytical value for quality control in wine and agricultural laboratories.

Learn More

You are looking for further Information?
Check out the link or scan the QR code for more details.

www.bruker.com/evoq-gc

References

[1] Bruker Application Note LCMS-136: *Determination of SVOCs in water samples using the Bruker µDROP method for the EVOQ GC-TQ MS/MS system*

[2] *Analytical quality control and method validation procedures for pesticide residues analysis in food and feed.* www.eurl-pesticides.eu/userfiles/file/EurlALL/AqcGuidance_SANTE_2019_12682.pdf

[3] *Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides.* data.europa.eu/eli/dir/2009/128/oj

[4] *Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.* data.europa.eu/eli/reg/2009/1107/oj

[5] *Regulation (EU) No 652/2014 of the European Parliament and of the Council of 15 May 2014,* data.europa.eu/eli/reg/2014/652/oj

[6] www.oiv.int/public/medias/6371/oiv-statistical-report-on-world-vitiviniculture-2018.pdf

[7] ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/

[8] *Regulation (EU) 2019/533 of 28 March 2019,* data.europa.eu/eli/reg_impl/2019/533/oj

For Research Use Only. Not for Use in Clinical Diagnostic Procedures.

Bruker Daltonics GmbH & Co. KG

Bremen · Germany
Phone +49 (0)421-2205-0

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com – www.bruker.com