INSTRUMENTAL ANALYSIS OF TRIFLUORO-ACETIC ACID (TFA) USING THE QuPPe METHOD

Ann-Kathrin Schäfer[1], Eric Eichhorn[1], Cristin Wildgrube[2], Michelangelo Anastassiades[1]

[1]EURL-SRM hosted at CVUA Stuttgart, Residues and Contaminants, Fellbach, Germany
[2]CVUA Stuttgart , Residues and Contaminants, Fellbach, Germany

E-Mail: ann-kathrin.schaefer@cvuas.bwl.de

Introduction

Trifluoroacetic acid (TFA) is a breakdown product of numerous industrial chemicals and pesticide active substances containing a trifluoromethyl moiety. Difluoroacetic acid (DFA) is a metabolite of the pesticide active substance flupyradifurone, which is approved for applications in the EU. Being small and highly polar molecules, TFA and DFA are suitable for being implemented into the QuPPe method (EN 18032) [1,2]. Currently, several analytical columns can be used for the determination of polar anionic pesticides and are therefore also potentially suited for the analysis of TFA and DFA. However, analysis of TFA is challenged by three major obstacles: **(a)** contamination of solvents and consumables; **(b)** the difficulty to find blank samples for some types of commodities; and **(c)** the lack of suitable and sensitive mass transitions (MRMs), making it difficult to meet the SANTE identification criteria at low levels [3]. Here, an overview on the available QuPPe-based methods for the analysis of TFA and DFA is given and approaches to handle analytical difficulties are described.

Analytical Methods and Experimental

QuPPe extracts of various food samples were measured using methods M1.7, M1.9, M1.10 and M1.12; each employing a different analytical column (see **Table 1)** and all connected to a Sciex 5500 [2]. For measuring DFA and TFA, the optimized MRM tune data was added to these methods without adjustments of the hardware. In addition, method M11 involving IC separation and MS/MS on a Sciex 7500⁺ was used. Method M11 was also used to generate the TFA data presented in PM-12 but using a Sciex 6500⁺ instrument. The following MRMs (*m/z)* were targeted: **DFA** 95→51; 95→95 and **TFA**: 113→69; 113→113; 69→19; 113→19. To compare the performance of the five methods, QuPPe extracts of 45 fruit and vegetable samples, collected in 2026 in Germany, were analysed. In a parallel project (see **(c)** & PD-14), the main MRM of TFA was also measured at different collision energies (ColEn) to evaluate the practical use of the ColEnDIR identification approach.

Table 1: Overview of QuPPe-methods tested for TFA/DFA analysis, measurement conditions see [2].

Method [2]	Approach	Column name	Run time in min
M9*	HILIC	Thermo Acclaim Trinity P1	10
M1.7	HILIC	Waters Anionic Polar Pesticides	23
M1.9	HILIC	Restek Polar X	17
M1.10	HILIC	Sielc Obelisc N	13
M1.12	RP**	Phenomenex Luna Polar Pesticides	10
M11	IC	Thermo AS19	30 or 22

* original method, employing ion mobility separation (Selexlon); not tested in this project
** with embedded polar groups

Analytical Challenges

(a) Contamination

Being a persistent breakdown product of many industrial chemicals, TFA is quasi ubiquitous in chemicals and solvents used in laboratories and potentially also leaches from instruments. It is therefore important to regularly check the used chemicals and consumables, e.g. by reagent blanks. Relevant levels of TFA were observed by the EURL-SRM e.g. in formic acid of "high purity grade" [2].

(b) Lack of "residue-free" samples for validation

The unavailability of TFA "residue-free" samples makes it difficult to validate at a desired low level and set an LOQ that covers the vast majority of the levels in samples due to the 30% criterion regarding the background. To circumvent this inability, the SANTE guidelines (v2026) give recommendations on how to establish an LOQ in Appendix A [3]. For this, the sample with the lowest TFA background level should be subjected to replicate analyses and assessed for repeatability and identification criteria (**Fig.1**). Low-level recovery experiments are conducted by spiking at a level ≥3 times the background (**Fig.1**).

(c) Lack of suitably sensitive mass transitions

With the molecular masses of TFA and DFA being comparably low, only one proper MRM is available for MS/MS measurement; TFA *m/z* 113→69 and DFA *m/z* 95→51. For TFA, the MRM based on the in-source fragment $[M\text{-}CO_2\text{-}H]^-$ as parent and fluoride as daughter ion (*m/z* 69→19) and the MRM 113→19 were also measured with decent sensitivity on high-end instruments (Sciex 6500⁺ and 7500⁺), but using older generation MS instruments (here tested: Sciex 5500) sensitivity was very limited, see PD-14.

Results and Discussion

DFA was present at measurable levels in four samples, with all tested methods providing comparable results (ranging between 0.015 and 0.22 mg/kg using M1.7). Identification of DFA in these samples was not a problem with any of the methods. In the case of **TFA**, the performance of the above measurement methods was compared by analyzing extracts of 45 samples. For identification, the SANTE criteria were applied (ion ratio, RT, ILIS, peak shape) [3] and additionally a cutoff was set when S/N of the qualifier (113→113) was <30. Among the four LC-MS/MS methods, M1.7 achieved the highest identification rate (**Fig.2**, blue). The overall highest TFA identification rate was achieved by M11 (using the highly sensitive Sciex 7500⁺), where three qualifier MRMs were measurable in all cases. Using the ColEnDIR approach, identification by the four LC-methods employing the older generation MS/MS became possible at much lower levels, resulting in a much higher number of samples with TFA being identified (**Fig.2**, grey). Therefore, the resulting median of samples with identified TFA shifted towards lower values when using ColEnDIR (**Fig.3**). Overall, the identification rate of M1.7 (on a Sciex 5500) combined with ColEnDIR was comparable with that achieved by M11 (on a Sciex 7500⁺) with the traditional identification criteria (see **Fig.2** and **Fig.3**). Furthermore, RT shifts were observed using M1.9, while M1.12 was struggling with *m/z* 113→113.

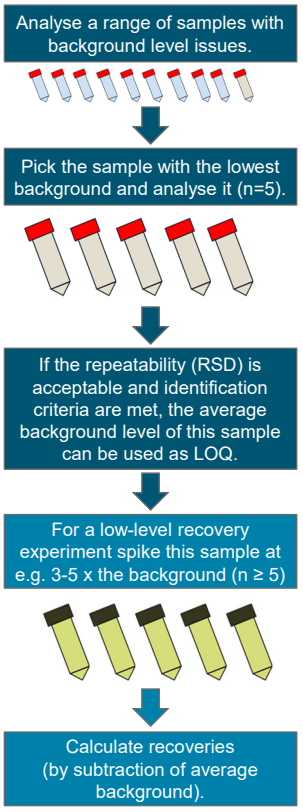


Fig.1: Validation approach in case of lack of „residue-free" ‚blank' samples according to the SANTE guidelines [3].

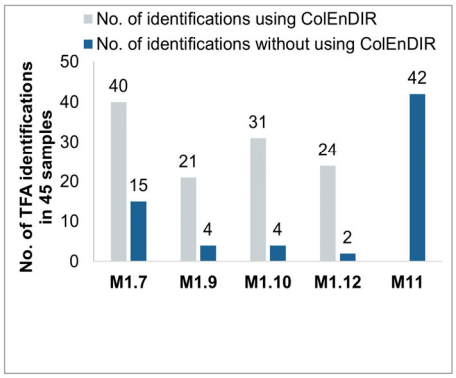
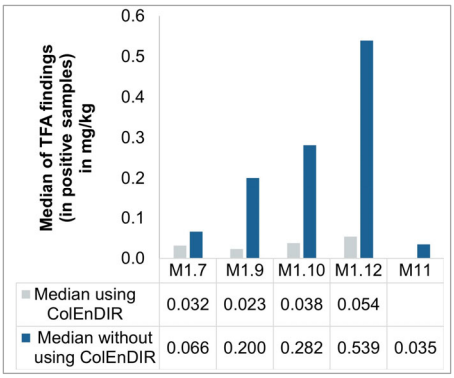


Fig.2: Number of TFA identifications in 45 different fruit and vegetable samples using M1.7, M1.9, M1.10 or M1.12 with (grey) / without (blue) ColEnDIR (Sciex 5500) and M11 without ColEnDIR (Sciex 7500⁺).



	M1.7	M1.9	M1.10	M1.12	M11
Median using ColEnDIR	0.032	0.023	0.038	0.054	
Median without using ColEnDIR	0.066	0.200	0.282	0.539	0.035

Fig.3: TFA median in positive fruit and vegetable samples using M1.7, M1.9, M1.10 or M1.12 with (grey) / without (blue) ColEnDIR (Sciex 5500) and M11 without ColEnDIR (Sciex 7500⁺).

Summary

The analytes TFA and DFA were successfully implemented into existing chromatographic methods for anionic polar pesticides and metabolites described in the QuPPe method [3]. The sensitivity was increased by using ColEnDIR to support identification especially at low levels of TFA. The sensitivity of M1.7 and a Sciex 5500 using ColEnDIR was comparable to the sensitivity of M11 and Sciex 7500⁺, which represents ‚high-end' instrumentation.

LITERATURE

[1] CEN EN 18032:2025: QuPPe.
[2] Anastassiades *et al.* QuPPe-PO-Method V12.3, see www.quppe.eu.
[3] Analytical quality control and method validation procedures for pesticide residues analysis in food and feed – SANTE 11312/2021 v2026.

EPRW 2026
PD-16

Co-funded by the European Union

Baden-Württemberg