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Development and Validation of a GC-ECD Method for the Determination of Trifluralin in Acetonitrile with Uncertainty Estimation

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The use of trifluralin herbicide has raised concerns due to its persistence in the environment and potential toxicological impacts on human health and ecosystems. The aim of this study was to develop and validate a simple and reliable method for determining trifluralin residues in acetonitrile using gas chromatography with an electron capture detector (GC-ECD). The method was developed using internal standard calibration and validated in accordance with international guidelines. Validation parameters included LOD, LOQ, selectivity, linearity, precision, accuracy (recovery and bias) and measurement uncertainty. The method demonstrated high selectivity with no interfering peaks at the trifluralin retention time. A strong linear relationship was observed in the concentration range of 4-11 µg/g with a correlation coefficient (R^2) of 0.9996. The precision, expressed as relative standard deviation (%RSD) was found 3.84% across all levels. Recovery values ranged from 98.45% to 100.06%, with % bias between 0.06% and 1.55% confirming the method accuracy. The expanded uncertainty was estimated to be 9.86% primarily influenced by sample and internal standard (IS) peak areas. The validated GC-ECD method provides a reliable, precise and accurate approach for quantifying trifluralin residues in environmental and food samples. It is suitable for routine monitoring, regulatory applications and risk assessments in agricultural and environmental contexts.

Keywords: Trifluralin, GC-ECD, Calibration, Validation, Uncertainty.

INTRODUCTION

Pesticides are widely applied in agriculture to manage pests, diseases, weeds and other plant pathogens aiming to minimize crop losses, prolong the shelf life of food products and maintain high quality yields [1-3]. However, the excessive use of pesticides can negatively impact the environment contaminating water, soil, air and disrupting the ecological balance in addition to posing significant risks to human health [4,5]. Trifluralin is a widely used herbicide belonging to the dinitroaniline class and commonly applied as a pre-emergent herbicide to control annual grasses and broadleaf weeds. Due to its chemical stability and semi volatile nature, residues of trifluralin may persist in environmental media such as soil, water and food products for an extended period after application. Its presence in food is of particular concern as studies have shown potential toxic effects including hepatotoxicity, nephrotoxicity, endocrine disruption and possible carcinogenicity especially in animal models [6]. Because of these risks, the use of trifluralin has been banned by the European Union in 2008 and the EU Regulation (EC) No 396/2005 set a default maximum residue limit (MRL) of 0.01 mg/kg for trifluralin [7]. As a result, accurate measurement of trifluralin concentrations in food and environmental samples has become a top priority for food safety laboratories, pesticide monitoring programs and producers engaged in international trade.

Analytical methods must be capable of detecting trifluralin at or below 0.01 mg/kg with validated accuracy, precision. Several analytical techniques have been developed for the determination of trifluralin such as GC-FID, GC-ECD and GC-MS as well as HPLC-UV [8]. Raeppel *et al.* [9] developed a GC-MS multiresidue method for analyzing 31 pesticides, including trifluralin in indoor and outdoor air using Tenax® tubes and SPME fibers. Trifluralin did not require derivatization and was unaffected by MtBSTFA allowing for simultaneous detection of both derivatized and non-derivatized compounds. The method is suitable for comprehensive atmospheric pesticide monitoring. A validated GC-ECD method was

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developed for analyzing trifluralin, atrazine and pendimethalin in fish using modified QuEChERS (quick, easy, cheap, effective, rugged and safe) with ultrasound assisted extraction and dispersive SPE. The method achieved good linearity (R^2 = 0.9973-0.9998), low LOD/LOQ (0.015-0.60 and 0.050-2.0 µg/kg) and high recovery (76.8%-94.6%) confirming its effectiveness for detecting trifluralin in fish [10].

A GC-MS/MS method was developed for the analysis of trifluralin and other herbicides in olive oil, employing acetonitrile/*n*-hexane extraction followed by Florisil clean-up. The method demonstrated linearity over the range of 1–500 µg/kg (R^2 > 0.996), with recoveries between 90% and 102% and reproducibility ranging from 8% to 11% [11]. The QuEChERS method has become a known approach in multi residue pesticide analysis due to its simplicity, high recovery rates and compatibility with both GC and LC methods [12]. Karasali *et al.* [13] developed and validated a multi-pesticide method for the quality control of commercial pesticide products containing alachlor, chlorpyrifos methyl, fenthion and trifluralin. They used a capillary gas chromatographic system with flame ionization detection (FID). The specificity, linearity, precision and repeatability of the method satisfied international acceptability criteria. Anastasiades *et al.* [14] have developed and validated a method for analysis of 25 multiclass pesticide residues and their metabolites in wheat grains using GC-ECD for quantification and GC-MS for confirmation. Sample preparation included extraction by acetonitrile and clean up by C-18, PSA and anhydrous $MgSO_4$. Limit of detection (LOD) of pesticides in wheat matrix varies from 0.002-0.06 µg/g and limit of quantitation (LOQ) from 0.004 to 0.2 µg/g. Mean recovery percentage of pesticides lies in the range of 79.77-128.04 with RSD below 16.35%.

Certified reference materials (CRMs) are an essential component in pesticide analyses because they provide traceability of the measurement results to the SI units through the calibration of measuring instruments [15]. They also help verifying accuracy of the measurement results and estimating measurement uncertainty. Without these materials, laboratories face challenges in ensuring comparability and reproducibility of results, particularly when operating near regulatory thresholds [16]. In this study, a method for the determination of trifluralin in acetonitrile using a GC-ECD system is developed. Calibration was carried out over the range of 4–11 mg/kg using a certified reference material (CRM) from the National Metrology Institute (NMI) of Australia, with aldrin employed as the internal standard. The method was validated by evaluating the limit of detection (LOD), limit of quantification (LOQ), selectivity, precision, accuracy (*via* recovery studies), linearity and measurement uncertainty.

EXPERIMENTAL

Acetonitrile (HPLC grade) was obtained from Merck, Darmstadt, Germany. The certified reference material (P1408), Trifluralin 99.9 ± 0.3%, was obtained from NMIA, Australia and aldrin (97%) was purchased from LGC-Dr Ehrenstorfer GmbH, Germany.

Analyses of trifluralin was performed by GC-ECD method using a Thermo-Fisher gas chromatograph instrument (Thermo-Scientific, Waltham, Massachusetts, USA). The chromatographic separation was performed using a TG5MS 30 m × 0.25 mm × 25 µm column (Thermo, USA). The column temperature was initially held at 100 ºC for 1 min. It was then programmed at 20 ºC/min to 180 ºC with no hold. After that, the temperature was increased at a rate of 5 ºC/min to 270 ºC. Finally, it was ramped at 20 ºC/min to 300 ºC and held for 2 min. The carrier gas was helium at 1.2 mL/min and 1 µL was injected splitless at 250 ºC. The instrument control, data acquisition and processing were measured by the Chremelone and Xcalibur software packages. A calibrated analytical balance of 220 g capacity and 0.01 mg resolution obtained from (Mettler Toledo), Switzerland was used for weighing.

Preparation of CRM stock solution: A stock solution of CRM in acetonitrile was prepared at a target mass fraction of 210 mg/kg in a 100 mL volumetric flask. A mass of 4.030 mg of CRM required for this preparation was calculated using eqn. 1 [17]:

$$x = \frac{m \times p}{m_{soln}} \quad (1)$$

where x is the mass fraction of stock solution (mg/kg); m is the mass of CRM (mg); p is the purity of CRM (%); and m_{soln} is the mass of CRM stock solution (kg).

The actual mass fraction of the stock solution was found to be 209.62 mg/kg. On the other hand, a stock solution of IS of mass fraction 55.59 (mg/kg) was prepared in acetonitrile.

Preparation of calibration solutions: A calibration range was designed from 6 points as: 4, 6, 7, 8, 9 and 11 mg/kg. The preparation of these calibration solutions, was carried out by dilution from the diluted CRM stock solution using eqn. 2 [18]:

$$x_1 \times m_1 = x_2 \times m_2 \quad (2)$$

where x_1 = mass fraction of the CRM stock solution (mg/kg); m_1 = mass of the CRM stock solution required for dilution (mg); x_2 = mass fraction of the diluted CRM stock solution (mg/kg); m_2 = mass of the diluted CRM stock solution (kg).

An internal standard (IS) mass fraction of approximately 8 mg/kg was added to each calibration solution.

Preparation of unknown sample: A trifluralin sample prepared in acetonitrile was used for the development of a GC-ECD method for the determination of this pesticide. An initial screening was performed by running a full scan of the sample alongside a certified reference material (CRM) with a known concentration. The preliminary concentration was estimated to be approximately 5 mg/kg. Then, three portions of the received sample weighing 1038.09, 1030.81 and 1024.98 mg were taken. To each portion of the unknown sample, 5 mg/kg of IS was added and then, the prepared samples were measured.

RESULTS AND DISCUSSION

Method development: A selective GC-ECD method was developed for the determination of trifluralin in acetonitrile. Fig. 1 shows a chromatogram of an unknown sample in which trifluralin was detected at 7.235 min and the internal standard, aldrin at 11.420 min. The chromatogram shows a good separation of the analyte with a total run time of 12.5 min, making the method both efficient and cost-effective.

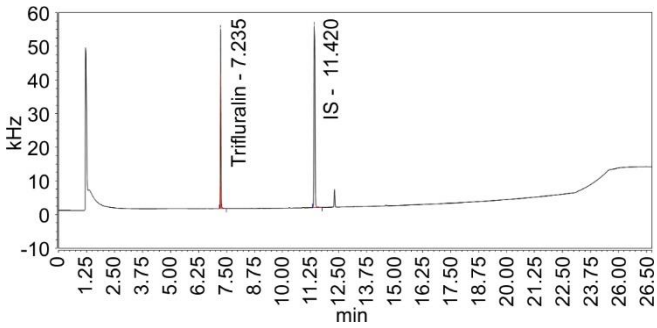

Fig. 1. GC-ECD chromatogram of trifluralin

After the method development, six calibration solutions were injected. Both the concentration ratio (C_{CRM}/C_{IS}) and the area ratio (A_{CRM}/A_{IS}) were calculated as summarized in Table-1.

TABLE-1
THE CONCENTRATION RATIO AND THE AREA RATIO DATA OF CRM AND IS FOR GC-ECD CALIBRATION

C_{CRM}	C_{IS}	C_{CRM}/C_{IS}	A_{CRM}	A_{IS}	A_{CRM}/A_{IS}
3.834	8.068	0.47527	0.37577	1.50093	0.24875
6.028	8.021	0.75156	0.45637	1.31433	0.34656
7.455	7.979	0.93435	0.53310	1.30860	0.40734
7.995	7.975	1.00250	0.56440	1.30690	0.43184
8.756	8.011	1.09290	0.66080	1.42637	0.46264
11.253	8.007	1.40545	0.69553	1.23370	0.56366

A calibration curve was then obtained by plotting these two parameters as shown in Fig. 2.

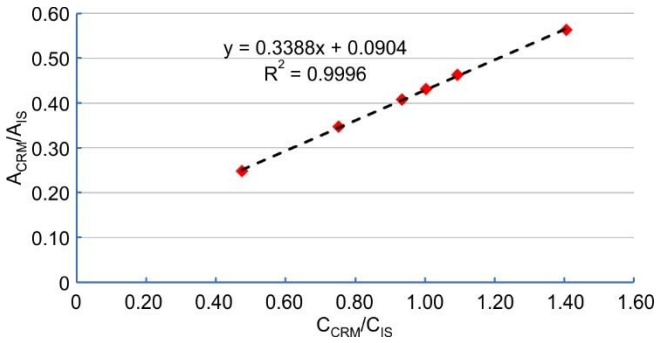

Fig. 2. GC-ECD calibration curve using trifluralin CRM

The resulting linear regression equation is: $y = 0.3388x + 0.0904$; where y represents the area ratio (A_{Un}/A_{IS}) and x represents the mass fraction ratio (C_{Un}/C_{IS}). The mass fraction of the unknown sample can thus be determined using eqn. 3 [19]:

$$C_{Un} = \frac{\left(\frac{A_{Un}}{A_{IS}} - b\right) \times C_{IS}}{a} \quad (3)$$

where A_{Un}/A_{IS} = area ratio; b = intercept; C_{IS} = mass fraction of the internal standard added to unknown sample and a = slope of the calibration line.

Method validation: Validation of the method under study has been carried out according to the EURACHEM Guide [17]. The validation covered the selectivity, LOD, LOQ, precision, accuracy and linearity.

Selectivity: The selectivity of the method refers to its ability to separate and detect the target compound without interference from other substances. As shown in Fig. 2, trifluralin appeared at a retention time of 7.235 min, while aldrin appeared at 11.420 min and no other peaks were observed at these retention times, which confirms the selectivity of the method.

LOD and LOQ: The LOD and LOQ were determined by a signal-to-noise ratio of 3:1 and 10:1 and they were estimated to be 3 µg/kg and 7 µg/kg, respectively.

Precision: Precision is a critical parameter for evaluating the performance of analytical methods. According to the International Vocabulary of Metrology (VIM), precision is defined as the closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions [15]. According to the guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed, the within-laboratory reproducibility (RSD) should be ≤ 20% [20]. In this study, precision was assessed through repeatability and intermediate precision experiments. Three blind samples (S_1-S_3) of identical concentration were prepared and analyzed using GC-ECD on different days by three different analysts. The measured mass fractions are summarized in Table-2.

TABLE-2
PRECISION RSD% DATA OF THE METHOD USING THREE UNKNOWN SAMPLES

	S_1	S_2	S_3
Mass fraction (mg/kg)	5.74	5.61	5.62
	5.34	5.89	5.75
	5.50	5.70	5.74
	5.64	5.49	5.40
	5.39	5.50	5.58
	5.78	5.53	5.45
	5.49	5.83	5.56
	5.74	5.82	5.52
	5.44	5.43	5.40
$\bar{x}$	5.56	5.64	5.56
Grand $\bar{x}$		5.59	
SD	0.17	0.17	0.13
Average SD		0.16	
MS-between groups		0.02396	
MS-within groups		0.02198	
(σ_b) intermediate precision		0.15	
SD of precision		0.21	
RSD%		3.84	

The standard deviation of the mean of each sample was calculated and the average SD (0.16) was used to represent the repeatability of the method. To evaluate intermediate precision, the between-group standard deviation (σ_b) was calculated by eqn. 4 using the $MS_{between}$ and MS_{within} from the one-way ANOVA, where n equals 9 and was found to be 0.15 (Table-3) [21].

$$\sigma_b = \sqrt{\frac{MS_{between} - MS_{within}}{n}} \quad (4)$$

The overall standard deviation of precision ($SD_{precision}$) was determined as the repeatability standard deviation (SD_{rept}) and

TABLE-3
ANOVA ONE-WAY DATA FOR THE PRECISION ANALYSIS RESULTS

Source of variation	SS	df	MS	F	P-value	F crit
Between groups	0.04792	2	0.02395	1.089	0.35	3.354
Within groups	0.59347	27	0.02198			
Total	0.64139	29				

the between-series standard deviation (σ_b). It was calculated by eqn. 5 and was found to be 0.21. Then, the relative standard deviation (%RSD) was calculated using eqn. 6 giving a small value of 3.84%. This result confirms that the method exhibits a very good precision since the %RSD is well below the commonly accepted threshold of ≤ 20% for pesticide residue analysis.

$$SD_{precision} = \sqrt{(SD_{rept})^2 + (\sigma_b)^2} \quad (5)$$

$$RSD\ (\%) = \frac{SD_{precision}}{x} \times 100 \quad (6)$$

Recovery and bias: In this study, the apparent recovery was evaluated using three certified reference material (CRM) samples with concentrations of 3.83, 7.46 and 11.25 mg/kg [21]. These concentrations were selected to represent the lower, middle and upper ranges of the calibration curve, allowing for an assessment of accuracy across the entire measurement range. Each sample was measured three times and the results are shown in Table-4.

TABLE-4
RECOVERY AND BIAS OF THE METHOD USING 3 CRM SAMPLES

CRM (mg/kg)	3.83	7.46	11.25
Measured mass fraction (mg/kg)	3.96	7.37	11.17
	3.71	7.54	11.28
	3.64	7.49	11.1067
Average	3.77	7.46	11.18
% Recovery	98.45	100.06	99.42
Bias (mg/kg)	-0.059	0.0045	-0.065
% bias	1.55	0.060	0.58

The apparent recovery was calculated by dividing mean of the measured mass fraction values by the certified value of the CRM and multiplying by 100, in accordance with eqn. 7. The resulting recovery values were 98.45%, 100.06% and 99.42% for the 3.83, 7.46 and 11.25 mg/kg samples, respectively [22]. These values indicate very good recovery across all levels reflecting a reliable measurement method.

$$Recovery\ (\%) = \frac{\bar{x}}{x_{ref}} \times 100 \quad (7)$$

Given the intrinsic relationship between bias and recovery, bias was also evaluated. It was calculated as an absolute value by eqn. 8 and as a percentage by eqn. 9 [22]. The calculated bias percentages were 1.55%, 0.060% and 0.58% for the 3.83, 7.46 and 11.25 mg/kg samples, respectively.

$$bias = \bar{x} - x_{ref} \quad (8)$$

$$bias\ (\%) = \frac{\bar{x} - x_{ref}}{x_{ref}} \times 100 \quad (9)$$

These results demonstrate that bias decreases with increasing mass fraction, being more pronounced at the lower mass fraction level and minimal at the highest. This trend is typical in trace analysis and suggests greater measurement uncertainty at low concentrations, which should be considered when interpreting results near the method detection limits.

Linearity: The linearity of the method was evaluated by plotting the residuals of the calibration points around an axis of zero as it can be seen from Fig. 3 in accordance with the IUPAC recommendation [23]. The random distribution of the residuals around zero indicates absence of the systematic error, supporting the assumption of linearity across the tested concentration range. Furthermore, the calibration yielded a linear regression equation of $y = 0.3388x + 0.0904$ with a coefficient of determination (R^2) of 0.9996. This high R^2 value confirms the excellent linear relationship between the mass fraction of trifluralin and the detector response demonstrating the method strong linear performance.

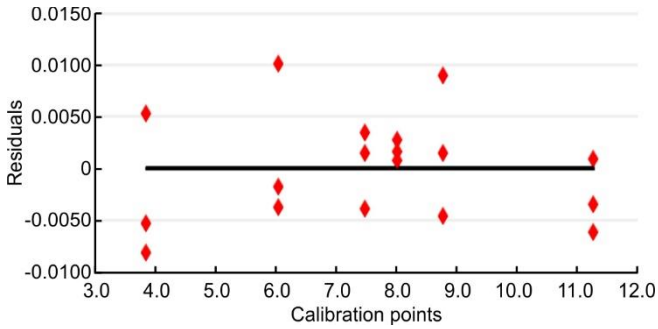

Fig. 3. Residuals of the calibration points of GC-ECD by trifluralin CRM

Uncertainty of measurements: Estimation of the measurement uncertainty is one of the fundamental technical requirements of ISO/IEC 17025 for calibration and testing laboratories and serves as the basis for applying the decision rule regarding conformity. Uncertainty estimation was carried out in accordance with ISO GUM and EURACHEM/CITAC Guide [24,25]. Both standards require a mathematical model for calculating the measurand and identifying the sources of uncertainty. In case of trifluralin determination, the measurand has been calculated using eqn. 3. This model allows for the identification of explicit sources of uncertainty, including: area of the unknown, area of the internal standard (IS), slope of the intercept, concentration of IS and slope of the calibration curve. From the analytical procedure, other implicit sources of uncertainty can be detected. These are: mass of unknown sample and CRM calibration solution, purity of CRM, purity of IS and calibration solution. Both explicit and implicit sources of uncertainty are shown as a fishbone structure in Fig. 4. Estimation of each source of uncertainty and the combined standard uncertainty as well as the expanded uncertainty is described below.

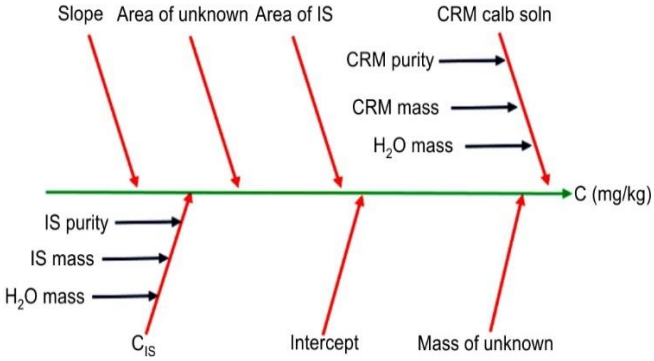

Fig. 4. Fishbone graph of the uncertainty sources

Uncertainty of explicit sources

Uncertainty of area of unknown sample (repeatability) (u_{rept}): The uncertainty of the area of unknown was calculated by dividing the standard deviation by √n, where n = 9 according to eqn. 10 and was found to be 0.011.

$$u_{rept} = \frac{SD}{\sqrt{n}} \quad (10)$$

Uncertainty of area of internal standard (u_{IS}): The uncertainty of the area of IS was also calculated by dividing the standard deviation by √n according to eqn. 9 and was found to be 0.029.

Uncertainty of slope (u(a)): For calculation of the uncertainty of slope (a), the standard deviation of regression, S was calculated first using eqn. 11:

$$S = \sqrt{\frac{\sum_{i=1}^{N}(y_i - b - ax_i)^2}{N-2}} \quad (11)$$

where N is the number of measurements in the calibration process; y_i is the area ratio; b is the intercept; a is the slope and x_i is the mass fraction of CRM calibration solutions.

The calculated S was then used to calculate the uncertainty of slope, u(a) using eqn. 12:

$$u(a) = \sqrt{\frac{S^2}{\sum_{i=1}^{n}(x_i - \bar{x})^2}} \quad (12)$$

where S = standard deviation of regression; x_i = mass fraction of the CRM and $\bar{x}$ = average mass fraction of CRM of calibration solutions.

Uncertainty of intercept (u(b)): Uncertainty of the intercept was calculated using eqn. 13. The standard deviation of:

$$u(b) = \sqrt{\frac{S^2\sum_{i=1}^{n}x_i^2}{n\sum_{i=1}^{n}(x_i - \bar{x})^2}} \quad (13)$$

regression S, uncertainty of slope u(a) and of intercept, u(b) were found to be: 0.0049, 0.0040 and 0.0023, respectively.

Uncertainty of mass fraction of IS [u(C_{IS})]: A stock solution of IS was prepared as 55.59 mg/kg based on eqn. 14:

$$C_{IS} = \frac{m \times p}{m_{soln}} \quad (14)$$

where C_{IS} = mass fraction of IS stock solution (mg/kg); m = mass of IS (mg); p = purity of IS (%) and m_{soln} = mass of IS stock solution (kg).

The uncertainty of IS stock solution was then calculated according to eqn. 15 and will be used in eqn. 17.

$$u_c(IS_{stock}) = \sqrt{\left(\frac{u_p}{p}\right)^2 + \left(\frac{u_{m_{IS}}}{m_{IS}}\right)^2 + \left(\frac{u_{m_{Solv}}}{m_{solv}}\right)^2} \quad (15)$$

The mass fraction of the IS (C_{IS}) added to the unknown sample was prepared by dilution from the IS stock solution, according to eqn. 16:

$$C_2 = \frac{C_1 \times m_1}{m_2} \quad (16)$$

where C_1 = concentration of the IS stock solution (mg/kg); m_1 = mass taken from the IS stock solution (mg); C_2 = concentration of IS to be added to the sample (mg/kg); m_2 = mass of solution of unknown (kg).

The uncertainty of the C_{IS} was then calculated using eqn. 17 based on the EURACHEM/CITAC Guide and was found to be 0.99 mg/kg.

$$u(C_{IS}) = C\sqrt{\left(\frac{u_{C_{stock}}}{C_{stock}}\right)^2 + \left(\frac{u_{m_{stock}}}{m_{stock}}\right)^2 + \left(\frac{u_{m_{soln}}}{m_{soln}}\right)^2} \quad (17)$$

Uncertainty of the implicit sources

Uncertainty of mass of unknown sample (u_m): The unknown sample was prepared by weighing a certain mass of trifluralin, IS and water using a calibrated analytical balance. The calibration certificate of the balance indicates that the standard uncertainty of mass measurement can be calculated from the relation 18, where R is the mass of trifluralin in µg and the factor 4.69×10^{-8} was quoted from the calibration certificate.

$$u_R = 4.69 \times 10^{-8} \times R \quad (18)$$

According to the EURACHEM/CITAC guide, this uncertainty contribution has to be counted twice, once for the tare and once for the gross weight. Thus, uncertainty of the mass of trifluralin was calculated, according to eqn. 19.

$$u(m_{triflu}) = \sqrt{2 \times (u_R)^2} \quad (19)$$

The uncertainty of the mass of unknown, u(m) is calculated by combining the uncertainties of weighing the mass of trifluralin, mass of IS and mass of solvent using eqn. 20 in which C_1, C_2 and C_3 are the sensitivity coefficients. Each C_i equals 1 since all uncertainty components are expressed in mg [25].

$$u_c(m) = \sqrt{\left(C_1 \cdot u_{m_{Triflu}}\right)^2 + \left(C_2 \cdot u_{m_{IS}}\right)^2 + \left(C_3 \cdot u_{m_{Solv}}\right)^2} \quad (20)$$

Uncertainty of CRM calibration solution ($u_{CRM\ CAL}$): A stock solution with a mass fraction of 0.017 mg/kg was prepared according to eqn. 1, and its associated uncertainty was calculated using eqn. 10. From this stock solution, six calibration solutions were prepared by serial dilution, following eqn. 21:

$$C_2(CRM) = \frac{C_1 \times m_1}{m_2} \quad (21)$$

where C_1 = mass fraction of the CRM stock solution (mg/kg); m_1 = mass taken from the CRM stock solution (mg); C_2 = mass

fraction of the CRM calibration solution (mg/kg); m_2 = mass of the CRM calibration solution (kg).

The uncertainty of the calibration solution was calculated according to the EURACHEM/CITAC Guide using eqn. 22:

$$u(C_{CRM}) = C\sqrt{\left(\frac{u_{C_{stock}}}{C_{stock}}\right)^2 + \left(\frac{u_{m_{stock}}}{m_{stock}}\right)^2 + \left(\frac{u_{m_{cal\ soln}}}{m_{cal\ soln}}\right)^2} \quad (22)$$

Incorporating the implicit sources, u_m and u_{CRM} into the model equation: The uncertainties associated with the mass of the unknown sample and the CRM calibration solution are considered implicit sources of uncertainty, as they are not explicitly included in the model represented by eqn. 5. Therefore, they were added to the uncertainty of C_{IS} as: u_m/m_{sample} and u_{CRM}/C_{CRM}, respectively. Thus, the modified uncertainty of the C_{IS} is expressed in eqn. 23.

$$u(C_{IS}) = C\sqrt{\left(\frac{u_{IS\ stock}}{C_{IS\ stock}}\right)^2 + \left(\frac{u_{m\ stock}}{m_{stock}}\right)^2 + \left(\frac{u_{m\ soln}}{m_{soln}}\right)^2 + \left(\frac{u_m}{m_{sample}}\right)^2 + \left(\frac{u_{CRM}}{C_{CRM}}\right)^2} \quad (23)$$

Combined standard uncertainty, u_c: For calculation of the combined standard uncertainty, u_c the sensitivity coefficients, $\delta c/\delta y_i$ (C_i) were obtained as in formulae (eqns. 24-28) by differentiation of the mathematical model in eqn. 3:

$$\frac{\delta C}{\delta A_{un}} = \frac{C_{IS}}{A_{IS} \times a} \quad (24)$$

$$\frac{\delta C}{\delta A_{IS}} = \frac{A_{un} \times C_{IS}}{a \times A_{IS}^2} \quad (25)$$

$$\frac{\delta C}{\delta b} = \frac{C_{IS}}{a} \quad (26)$$

$$\frac{\delta C}{\delta C_{IS}} = \frac{(A_{Un}/A_{IS}) - b}{a} \quad (27)$$

$$\frac{\delta C}{\delta a} = \frac{C_{IS}(A_{Un}/A_{IS} - b)}{a^2} \quad (28)$$

Using these partial derivatives, the combined standard uncertainty, u_c has been calculated by eqn. 29.

$$u_c = \sqrt{\left(\frac{\delta C}{\delta A_{un}} \cdot u_{A_{un}}\right)^2 + \left(\frac{\delta C}{\delta A_{IS}} \cdot u_{A_{IS}}\right)^2 + \left(\frac{\delta C}{\delta a} \cdot u_a\right)^2 + \left(\frac{\delta C}{\delta b} \cdot u_b\right)^2 + \left(\frac{\delta C}{\delta C_{IS}} \cdot u_{C_{IS}}\right)^2} \quad (29)$$

The calculated values of the uncertainty budget were compiled in Table-5 and the expanded uncertainty was calculated by multiplying the u_c in a coverage factor k = 2 at confidence level of 95% and was found to be 0.54 mg/kg or 10.34%.

To gain a better understanding of the uncertainty budget, the contributions were plotted against their respective magnitudes ($c_i \cdot u(y_i)$) as shown in Fig. 5.

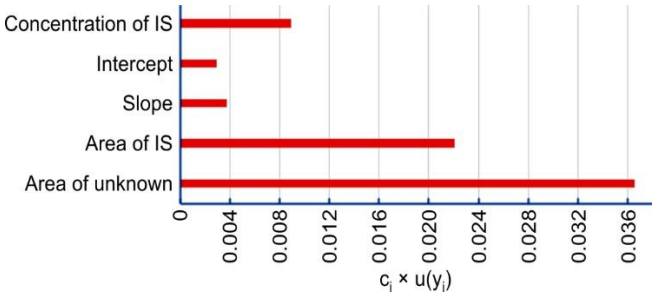

Fig. 5. Data based on different uncertainty contributions in measurement result of trifluralin

It is evident that the largest contribution comes from the peak area of the unknown sample, followed by the contribution of the internal standard peak area, then the internal standard concentration and finally the slope and *y*-intercept. Thus, it can be said that accurate and precise measurement of the sample and internal standard peak areas is critical to reducing the overall uncertainty of the method. Moreover, the slope and *y*-intercept of the calibration curve contribute the least to the overall uncertainty, indicating that the calibration method is stable and reliable.

Conclusion

A method for the determination of trifluralin residues in acetonitrile using GC-ECD has been developed and validated. It proven to be precise, accurate and highly selective, meeting the performance criteria required for pesticide residue analysis. The method exhibited excellent linearity (R^2 = 0.9996), reliable precision (RSD = 3.84%) and high percentage apparent recovery (98.45-100.06%) with a small bias confirming its robustness. The expanded measurement uncertainty of 9.86% remains within acceptable limits with regard to pesticide residue analysis, affirming the method reliability. These results demonstrate that the method is fit-for-purpose and can be confidently applied to routine monitoring of trifluralin in environmental and agricultural matrices, supporting both regulatory compliance and public health protection.

TABLE-5
UNCERTAINTY BUDGET DATA OF THE MASS FRACTION OF TRIFLURALIN BY GC-ECD

Quantity x_i	Estimate x_i	$u(y_i)$	Probability distribution	Sensitivity coefficient c_i	$c_i\ u(y_i)$
Area unknown	0.4365	0.011	Normal	16.8113	0.036565
Area IS	1.4082	0.029	Normal	-5.2109	0.022107
Slope (a)	0.34	0.0040	Normal	-15.3419	0.003709
Intercept (b)	0.0904	0.0023	Normal	-23.6736	0.002946
C_{IS}	8.12	0.146	Normal	0.6481	0.008894
Combined standard uncertainty, u_c				0.27	
Expanded uncertainty, Uexp	k = 2			0.54	
Expanded uncertainty, %				10.34	

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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