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Efficient Concurrent Analysis of Mesotrione, Atrazine, Benoxacor and S-Metolachlor in Herbicide Formulate: Developing and Validating a Reversed-Phased HPLC Method

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Abstract

An effective, simple and reliable simultaneous determination method for four active compounds, mesotrione, benoxacor, atrazine and S-metolachlor, in herbicide formulate was first-time developed and substantiated in current study using reversed-phased high performance liquid chromatography, following ICH guidelines. The chromatographic separation with fine resolution was conducted on a 5 µm C-18 column (L x id, 15 cm x 4.6 mm) using acetonitrile:water 70:30 (v/v) at 1.0 mL/min flow rate and wavelength 260 nm (UV detector). The retention times for mesotrione, atrazine, benoxacor and S-metolachlor were obtained as 1.90, 3.50, 3.95, and 5.10 min, respectively. Limits of detection of mesotrione, atrazine, benoxacor and S-metolachlor were found to be 2.57, 2.27, 1.28, and 1.32 µg/mL, whereas limits of quantitation were 7.80, 6.86, 3.86, and 4.01 µg/mL, respectively. The regression coefficients from calibration curves were > 0.998 for all studied pesticides. The method reproducibility was evaluated as intra-day precision (0.85–1.52 %RSD) and inter-day precision (0.67–1.81 %RSD). The method accuracy was estimated through inter-laboratory comparison among three laboratories. The developed method is quite rapid, inexpensive, robust, precise, accurate, linear and sensitive for the purpose of quality control check of mesotrione, atrazine, benoxacor and S-metolachlor simultaneously in herbicide formulations.

Keywords: Crop protection agents, Liquid chromatography, Method optimization, Robustness, UV detector, System suitability

Introduction

Pesticides are vital in modern agriculture for preventing plant diseases, reducing pests, and increasing product output and quality, when applied moderately and safely [1-2]. Agriculture is the biggest income generating sector in South Asia that is thus associated with high pesticides usage. Pakistan consumes 130,000 metric tons of pesticides annually, of which ninety percent are used on cotton, fruits, vegetables and rice [3]; China provides 91 percent of Pakistan's pesticide needs [4]. The market for pesticide is anticipated to grow to USD 349.5 million by 2025 from USD 220 million valued in 2019. Based on FAO (Food & Agricultural Organization of the United States) data, the usage of pesticides on arable

land of Pakistan, India and China (Hong Kong SAR/Taiwan Province) in 2021 was 0.38, 0.37, and 18.33/13.4 kg/hectare, respectively, showing that it is very low for Pakistan or India compared to neighbor country China [5]. The Pakistan's pesticide industry chiefly comprises 272 small-scale import dealers for selling products to end consumers [4]. Presently, the Department of Plant Protection from Ministry of National Food Security & Research in Pakistan is in charge of standardizing, importing and regulating pesticides and developing "Good Pesticides Application Practices" in accordance with FAO/WHO criteria owing to concernments about overuse, misuse and mishandling of pesticides [6].

Farmers rely on specific formulated pesticides that are selected based on the type of control of pest or mode of application. The accuracy of the stated amount or concentration of active ingredient for labeled pesticide formulation is crucial to meet the recommended dosages to guarantee the controlled use of these formulated pesticides. Inappropriately stated concentrations or the deviation from the claiming percentage of active ingredients within formulations or use of unregistered pesticide products could have adverse consequences. The use of a pesticide formulations having active component lesser than stated percentage may not sufficiently protect the crop from targeted pests, whereas the over-dose of active component might give the agricultural crop crossing the MRL (maximum residual limit) that could affect the quality and safety of resulting food for human consumption [1,7]. In a survey by Khan et al., the farmers of Sindh, Pakistan were found to be seriously exposed to pesticide risk because of inadequate knowledge [8].

Highly hazardous pesticides (HHPs) affect health of human, livestock and other non-target organisms acutely or chronically [6, 9-10]. Herbicides residues or their degraded products can bioaccumulate and biomagnify even in low concentrations with known teratogenic, carcinogenic and mutagenic effects on humans, leading to serious ailments of neurological system (Parkinson's disease, Alzheimer's disease and others), reproductive system, liver and kidney [1-2]. It has raised a worldwide concern about the pesticides presence and necessitates their continuous monitoring in different matrices [11]. It is necessary to develop reliable, efficient, rapid, precise and accurate methods to quantitatively analyze pesticide product formulations to ensure their quality, safety and efficacy [12-13]. The herbicides mesotrione, atrazine, benoxacor and S-metolachlor were selected for present study for their simultaneous determination in pesticide formulations.

Mesotrione (2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione), a β-triketone, has been an efficient selective herbicide for more than one decade to protect maize from broadleaf weeds and annual grasses as either pre- or post-emergence application [14-15]. Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine), a chlorinated triazine, is a popular pre-emergent selective herbicide to encounter grassy and broadleaf weeds on corn, sorghum and sugarcane [16-17]. S-Metolachlor [(*S*)-2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(1-meth oxypropan-2-yl)acetamide] belonging to the chloroacetanilide family is also a globally used pre-emergent selective herbicide for soybean, corn, maize, sugar cane and cotton [18]. It is among the most frequently applied herbicides in the US, where the estimated application of S-metolachlor in 2015 was 2.3 $\times 10^7$ kilograms [19], whereas in 2018 about 29% of the acres cultivated with soybeans and corn were treated with S-metolachlor [20]. Benoxacor [(*RS*)-4-dichloroacetyl-3,4-

dihydro – 3 – methyl - 2H-1,4-benzoxazine], a dichloroacetamide, is a safener usually paired with S-metolachlor in pre-emergent (spray on soil) application to protect the crops, mainly corn, from toxic effects of herbicides [19,21]. The MRLs (mg/kg) of mesotrione, atrazine and S-metolachlor for different crops are found as 0.01–0.05 (corn and maize), 0.05 (sorghum, sugarcane, maize), and 0.02–0.05 (corn, cotton, peanuts), respectively [11]. Wide use of these herbicides requires their quantitative analysis as active contents in formulations for controlled crop application.

Different methods, including electrochemical, spectrophotometric, biosensing, electrophoretic and chromatographic, i.e., liquid (LC) or gas (GC) being the most common, methods have been previously documented for the identification and quantification of herbicides in various matrices [11]. Some of the methods for the currently selected herbicides, analyzed previously either separately or simultaneously with metabolites or other herbicides, are indicated here: mesotrione, square wave voltammetry-clay modified glassy carbon electrode [22], reversed phase high pressure LC with diode array detector (RP-HPLC/DAD) [23-24], RP-HPLC/UV [25], LC-MS/MS, i.e., with tandem mass spectrometry [26] and ultra HPLC-MS/MS [27-28]; atrazine, electrochemical (boron–doped diamond electrode [29], nanocomposite sensor and nanofilm-surface plasmon resonance sensor) [27], HPLC/UV [30], LC/variable wavelength detector [27], LC/DAD [30,31], LC-MS/MS [32], GC with flame ionization detector (GC/FID) [33]; metolachlor, GC-MS [27], GC-MS/MS [34] and HPLC/UV [35]; and benoxacor, RP-HPLC/UV [36] and GC-nitrogen phosphorous detector [37]. However, no analytical method has been developed so far for simultaneously determining four selected herbicides (mesotrione, atrazine, benoxacor, and S-metolachlor) in pesticide formulations.

The herbicides selected are often combined with other weedicides in formulated preparations under different trade names (Syngenta®) to effectively and simultaneously manage weeds, crops, and soil through synergistic and phytotonic effects [14,38]. One such combination-formulated product available in the international market is Lexar EZ, which was utilized in the current study. Quantifying all active herbicides in formulations within a single injection would be less time-consuming, more cost-effective, and simpler than separate runs for each target herbicide.

To our knowledge, mesotrione, atrazine, benoxacor and S-metolachlor have not previously been analyzed simultaneously in combination pesticide formulations using HPLC. Therefore, an attempt was made to establish an efficient and economic RP-HPLC/UV method to simultaneously identify and accurately quantify mesotrione, atrazine, benoxacor and S-metolachlor in pesticide formulation, with the validation of developed method following ICH guidelines [39]. Fig. 1 provides chemical structures of studied pesticides. The proposed strategy offers technical support for pesticide formulated product quality control and pursues the direction of utilizing less harmful solvents with diminishing the volume of reagents.

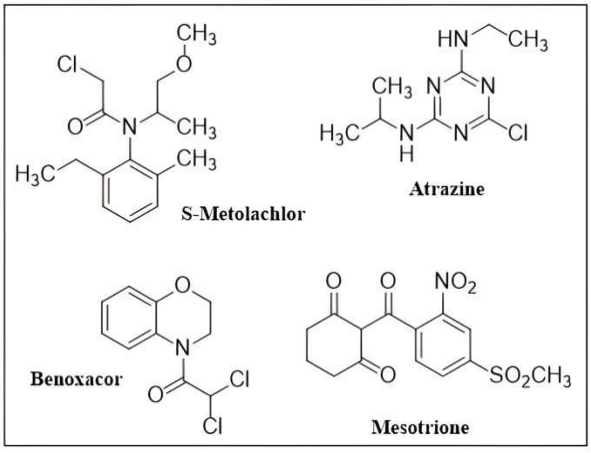

Figure 1. **Chemical structures of four selected pesticides**

Materials and Methods

Reagents and Chemicals

The organic solvent, acetonitrile (ACN), used herein was obtained from Merck (Germany) in HPLC grade. Distilled water after passing through deionizer (ELGA Cartridge Type C114) was utilized to prepare all solutions and mobile phase. The standard reference materials (mesotrione, Batch 492970, 99.5%; atrazine, Batch GBL2E26BB6, 97.1%; benoxacor, Batch AMS 248/4, 99.7%; and S-metolachlor, Batch MONSANTO 1453398, 98%) and pesticide formulated product (Lexar EZ) containing mesotrione, atrazine, benoxacor and S-metolachlor were obtained from Syngenta Pakistan Limited (Karachi, Pakistan).

Instrumentation

The experimental work was based on HPLC instrument (Shimadzu) attached to a UV-visible detector (SPD-10AV). The instrument included a rheodyne injector (20 µL loop), a bi-gradient delivering pump system for mobile phase, and C-18 column with following dimension for stationary phase: 5 µm, 4.6 mm x 15 cm. For integrating and analyzing the chromatographic data, chromatography software package (v. 1.7 DLL, S.N. 11-8199, Build 160502) utilized.

Optimization of Chromatographic Method and Experimental Conditions

The water/ACN mixture was utilized as a mobile phase targeting to optimized responses of analytes. To achieve best resolution or separation of analytes, different v/v ratios with mobile phase mixture were examined. For attaining shortest retention times of all the analytes (without effecting the shape and resolution of sample peaks), separation of analytes was assessed at various flow rates using mobile phase under isocratic mode. The C-18 column was installed at ambient temperature to separate analytes. To obtain the detection wavelength, different wavelengths in UV range between 210 and 290 nm were checked. The detection wavelength was finalized considering optimum experimental conditions where interferences of inert material of formulated product were minimum.

Standard Solutions Preparation and Calibration Curves

50 mL stock standard solutions of mesotrione, atrazine, benoxacor and S-metolachlor were individually made in ACN/water mixture (70:30 v/v) using 0.02 g pure analytical standard. The standard mixture solutions and further dilutions up to 1.25 µg/mL (for working standards) were made from stock solutions (400 µg/mL). Six (06) working standards of all four analytes were prepared in different ranges, i.e., 1.5–48 µg/mL (mesotrione), 1.25–40 µg/mL (benoxacor), 6–190 µg/mL (S-metolachlor) and 6–190 µg/mL (atrazine), to draw standard calibration curves (Fig. 2a). The analysis was done in triplicates.

Sample Solutions Preparation

The formulated sample solution was prepared at 0.05 g per 50 mL using diluent/mobile phase (ACN:water 70:30) and allowed to sonicate for complete dissolution of the analytes. The resulting sample solution was passed over Millipore membrane filter (0.45 µm) before injecting into the chromatographic column.

Results and Discussions

Composition of Mobile Phase

Various ratios of mobile phase solvents (water/ACN mixture) were monitored

for the optimization of mobile phase for good resolution to ensure well-separated peaks of four analytes with no overlapping. This allows for clear distinction among different analytes based on their retention times and accurate integration of peak areas, essential for accurate quantification of each component in the sample. Good chromatographic resolution also ensures the sensitivity and reliability of analytical results.

The mobile phase composition which provided the best resolution of peaks with shortest retention time (RT) was found to be 70:30 v/v; the data is summarized in Table 1. By decreasing ACN content in mobile phase composition, the retention time of S-metolachlor significantly shifted to the right. But on increasing the acetonitrile concentration more than 70% in the mobile phase, the asymmetry and tailing factor of mesotrione were disturbed due to affecting the interaction of the analyte with the stationary phase, associated with the change in solvent polarity, pH and ionic strength. Therefore, 70:30 ratio of mobile phase was suitably selected. Comparatively, Kaliyan & Tamilselvan report 20:80 water:ACN mobile phase composition as optimum for simultaneous RP-HPLC-UV identification of three similar herbicides (excluding atrazine) with somewhat higher RT of 3.8 min for mesotrione and 6.4 min for benoxacor while similar RT of 5 min for metolachlor at 230 nm on same flow rate [36].

Stationary Phase

The selection of stationary phase was accomplished between two different manufacturer's reversed phase columns (Discovery and C-18 Beckman column) with different specifications. Among two columns, the response to separate all four analytes was best with 5 µm Beckman column (15 cm L x 4.6 mm id) so it was selected for the current method.

Mobile Phase Flow Rate

The mobile phase flow rate significantly impacts the shape of the chromatogram, especially regarding peak asymmetry and the tailing factor, which evaluate chromatographic resolution and quality. At lower flow rates, the increased interaction time with the stationary phase can lead to greater tailing and asymmetry of peaks, particularly for polar or highly retained compounds. In contrast, higher flow rates reduce this interaction time, often decreasing tailing and asymmetry for weakly retained compounds. However, excessively high flow rates can cause peak broadening and poor resolution, as analytes have insufficient time to equilibrate between stationary and mobile phases. Hence, finding the optimal flow rate is essential for maintaining peak shape and resolution. The mobile phase flow rate was adjusted between 0.8–1.1 mL/min. It was found appropriate at 1.0 mL/min for fair resolution (Table 2).

Results indicate that flow rate deviation from 1.0 mL/min resulted in increased asymmetry and tailing factor of mesotrione and S-metolachlor; however, responses of rest of the analytes remained almost unaffected by alteration in the flow rate across 1.0 mL/min. Therefore, 1.0 mL/min flow rate was considered to be suitable for this method. In contrast, a mobile phase (methanol/water, 50%) flow rate of 0.8 mL/min remained optimum in a closely related previous simultaneous RP-HPLC-UV analysis of atrazine/simazine/mesotrione for vegetables/sediment/waters by Baranowska et al. [25].

Table 1. **Selection of mobile phase composition for simultaneous determination of herbicides mesotrione, atrazine, benoxacor and S-metolachlor.**

ACN: Water	Mesotrione			Atrazine			Benoxacor			S-Metolachlor		
	Assy.[a]	TF[b]	T_R, min[c]	Assy.[a]	TF[b]	T_R, min[c]	Assy.[a]	TF[b]	T_R, min[c]	Assy.[a]	TF[b]	T_R, min[c]
50:50	0.86	1.08	1.60	0.67	0.86	6.17	0.69	0.87	9.97	0.96	0.98	14.33
60:40	1.17	1.27	1.69	0.81	0.93	4.25	0.69	0.86	5.68	1.00	1.00	7.57
70:30	1.31	1.21	1.91	0.99	1.01	3.52	0.83	0.94	3.92	0.95	0.98	5.12
80:20	1.50	1.37	1.90	0.99	1.01	3.57	0.86	0.96	3.88	0.93	0.98	4.89

[a]Asymmetry. [b]Tailing factor. [c]Retention time.

Table 2. **Flow rate selection for simultaneous determination of herbicides mesotrione, atrazine, benoxacor and S-metolachlor.**

Flow rate, mL /min	Mesotrione		Atrazine		Benoxacor		S-Metolachlor	
	Assy.[a]	TF[b]	Assy.[a]	TF[b]	Assy.[a]	TF[a]	Assy.[a]	TF[a]
0.8	1.432	1.351	1.153	1.221	0.945	0.960	1.145	1.125
0.9	1.467	1.311	1.077	1.051	0.808	0.927	1.010	1.013
1.0	1.313	1.209	0.986	1.013	0.830	0.940	0.950	0.984
1.1	1.468	1.272	0.932	0.983	0.839	0.942	0.988	1.002

[a] Asymmetry. [b] Tailing factor.

Wavelength Selection

The detection wavelength was determined by UV scanning between 210 and 290 nm on HPLC program. Fig. 2b showing optimized chromatographic responses for four investigated pesticides depicts 260 nm as the detection wavelength. Although the responses on chromatograms of mesotrione, benoxacor and S-metolachlor were also uniform at wavelength 220 nm, atrazine showed a sharp decline in its response at 220 nm; therefore, 260 nm was considered as a detection wavelength, where relatively low chromatographic responses were observed but all of those were uniform [36]. A comparative chromatogram of standard and sample having peaks of all four analytes with good resolution and separation at optimized conditions is shown in Fig. 3.

Different testing parameters including system suitability, linearity, accuracy, precision and robustness were evaluated and discussed below with the aim of validating developed method for simultaneous analysis of mesotrione, benoxacor, S-metolachlor and atrazine, according to the ICH guidelines [39].

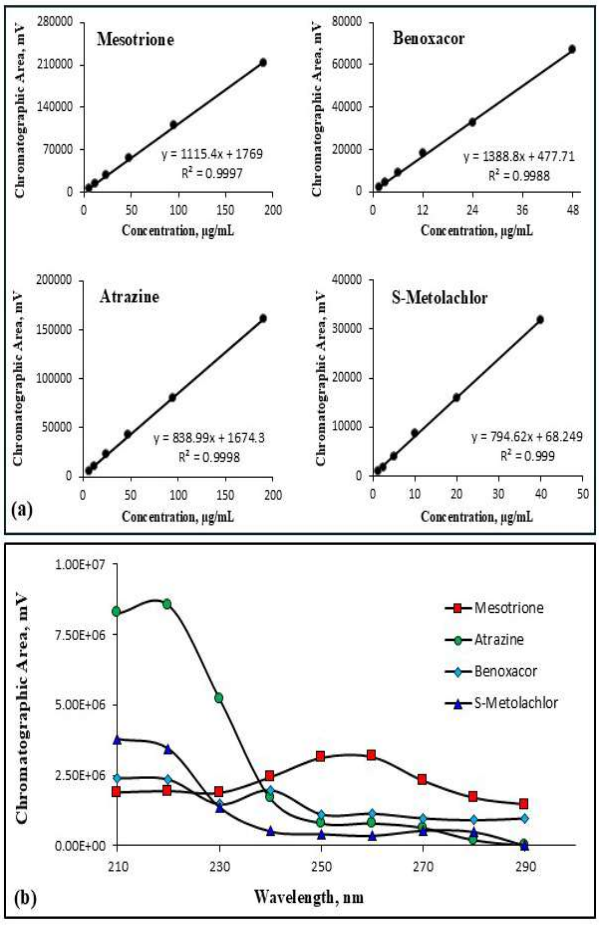

Figure 2. **(a) Calibration curves and (b) detection wavelength selection for four selected pesticides (Mesotrione, Benoxacor, Atrazine, and S-Metolachlor)**

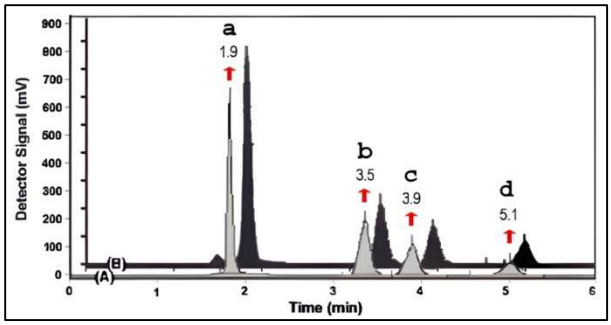

Figure 3. **A comparative HPLC chromatogram of standard (A) and sample (B) of studied herbicides: (a) mesotrione, (b) atrazine, (c) benoxacor and (d) S-metolachlor**

System Suitability Test

This test bears high significance in terms of checking the optimal performance, resolution and reproducibility of the chromatographic system. Various parameters including capacity factor, asymmetry, No. of theoretical plates (N), height equivalent of a theoretical plate (H) and half-width were calculated for the test and shown in Table 3. For a given column, the greater the value of N the greater is the number of ideal equilibrium stages in the system and the more efficient is the separation. The plate height (H) is equal to the column length (L) divided by N. A shorter H indicates more plates contained in a given L and a narrower solute peak with better resolution, translating to higher column efficiency. The half-width is directly related to the chromatographic system resolution. A smaller half-width typically indicates a narrower peak and that the system is effectively resolving the compounds with minimal dispersion, while larger half-width (broader peaks) can indicate poor efficiency or possible problems like column overload or mobile phase issues. The replicate analysis of simultaneous determination of mesotrione, atrazine, benoxacor and S-metolachlor provided precise results, which verifies the suitability of the chromatographic system for the developed method.

Table 3. **System suitability test for simultaneous determination of herbicides mesotrione, atrazine, benoxacor and S-metolachlor.**

Herbicide	Half-width	Asymmetry	Capacity factor	Efficiency (N)[a]	H[b]	Resolution
Mesotrione	0.047	1.625	1.403	8075.107	192.944	2.159
	0.045	1.652	1.414	8929.779	186.126	2.407
	0.047	1.667	1.426	8150.838	184.55	2.437
	0.033	1.673	1.432	8493.731	183.43	2.447
Atrazine	0.130	0.959	1.955	3767.232	31.519	1.500
	0.132	0.973	1.257	3618.497	31.504	1.424
	0.130	0.947	1.242	3675.196	31.673	1.467
	0.133	0.973	1.256	3483.731	30.987	1.447
Benoxacor	0.147	0.83	2.836	4082.972	28.560	2.139
	0.153	0.851	2.798	3688.887	28.510	2.076
	0.150	0.849	2.791	3822.256	29.540	2.113
	0.165	0.818	2.689	3172.262	24.512	2.011
S-Metolachlor	0.180	0.972	4.189	4576.205	27.267	3.648
	0.172	1.069	3.716	4982.763	28.430	3.667
	0.172	0.980	3.406	4886.456	27.880	3.601
	0.183	0.899	3.124	4312.334	24.604	3.349

[a] No. of theoretical plates. [b] Height equivalent to the theoretical plate.

Linearity

Linearity of mesotrione, atrazine, benoxacor and S-metolachlor was determined through calibration curves of peak area vs. concentration range of each pesticide i.e., 6–190 µg/mL (mesotrione, atrazine), 1.5–48 µg/mL (benoxacor) and 1.25–40 µg/mL (S-metolachlor). The regression coefficient (R^2) values for these calibration curves were > 0.998, possessing good linearity (Fig. 2a). The observed linearity ranges for individual herbicides vary (narrower or broader) compared to respective literature values due to difference in matrix and applied HPLC-UV chromatographic conditions [24,25,36].

Limit of Detection and Quantification

ICH guidelines were followed to determine the LOQ and LOD, the limit of quantification and limit of detection, respectively, for the suggested method. These values were computed from standard deviations of the chromatographic responses among results (σ) and corresponding slopes (S). The σ values were determined using calibration curves. LOQ and LOD were estimated using formulae given below at S/N ratio ten-fold and three-fold relative to base line, respectively.

$$\mathrm{LOQ} = \frac{10\,\sigma}{S} \quad (1)$$

$$\mathrm{LOD} = \frac{3.3\,\sigma}{S} \quad (2)$$

LOD value of mesotrione, atrazine, benoxacor and S-metolachlor was found to be 2.57, 2.27, 1.28, and 1.32 µg/mL, respectively, whereas LOQ values were 7.80, 6.86, 3.86, and 4.01 µg/mL, respectively.

Precision

To check method precision, the repeated analyses of sample containing all four active pesticide analytes were performed within a day (n = 3) and for three days, and the precision is reported as intra-day and inter-day, respectively (Table 4). The percent RSD (relative standard deviation) corresponding to intra-day precision observed at 0.85–1.52, whereas for inter-day precision the % RSD ranged 0.67–1.81. The average percent recoveries for all four analytes (calculated as a ratio of the amount of analyte detected to the actual amount of analyte present in the sample mixture) were between 99.16 and 100.34 for intra-day precision, and between 98.50 and 101.17 for inter-day precision (Table 4). The slightly overestimated (above 100%) percent recovery values in some cases might be attributed to technical factors, including instrumental (detector sensitivity/baseline noise) or matrix effects. However, the percent recovery data for the four analytes remained within the standard acceptable limits (80–120%). The %RSD for two precision tests was found to be < 2, confirming a good (acceptable) precision of method for simultaneous analysis of pesticides in formulations [24].

Table 4. **Method precision (inter-day and intra-day).**

Herbicide	Intra-day analysis, n = 3				Inter-day analysis, n = 3		
	Actual, % w/w	Result, % w/w	RSD, %	Average recovery, %	Result, % w/w	RSD, %	Average recovery, %
Mesotrione	2.40	2.38	1.18	99.16	2.41	0.67	100.46
Atrazine	19.00	19.01	1.30	100.03	19.34	1.81	101.80
Benoxacor	2.00	2.00	1.52	99.93	1.97	0.74	98.50
S-Metolachlor	19.00	19.07	0.85	100.34	19.22	0.97	101.17

Accuracy

The accuracy of proposed method was inspected through Inter-laboratory comparison (ILC). For this purpose, the pesticide formulation comprising mesotrione, atrazine, benoxacor and S-metolachlor was also assessed in two more labs: a) PCSIR (Pakistan Council of Scientific & Industrial Research – Karachi), b) Syngenta Pakistan Limited – Karachi. The in-house Food Quality & Safety Research Institute, Pakistan Agriculture Research Council (FQSRI, PARC) analytical results were compared to results of other two laboratories. Table 5 shows good accuracy of the method to quantitatively analyze mesotrione, benoxacor, atrazine and S-metolachlor simultaneously in formulated pesticide products. Analytical results obtained from all participating laboratories were subjected to determine Z-score values (Fig. 4). Z-score values are used to interpret the reliability of the laboratory analysis as $2 < |Z| \leq 3$, questionable; $|Z| \leq 2$, gratifying; and $|Z| \geq 3$, de-gratifying. Z-score value for each lab (Zi) was determined using given formula:

$$Zi = \frac{(Xi - \bar{X})}{S} \quad (3)$$

Xi, $\bar{X}$ and S in the above equation represent estimated result from a lab, average of results from all labs and standard deviation amongst results, respectively.

The values of Z-score of all the pesticides obtained from proposed method were found to be less than 2, proving gratifying results and ensuring the reliability and reproducibility of the developed method [40].

Table 5. **Comparison of inter-laboratory tests for formulation of mesotrione, atrazine, benoxacor and S-metolachlor.**

Herbicide	Lab 1[a]		Lab 2[b]		Lab 3[c]	
	Result, % w/w	RSD, %	Result, % w/w	RSD, %	Result, % w/w	RSD, %
Mesotrione- 2.44%	2.48	2.04	2.51	1.83	2.58	1.84
Atrazine – 18.61%	18.62	1.22	18.75	0.26	18.59	0.43
Benoxacor – 0.39%	0.38	0.86	0.39	3.13	0.39	1.39
S-Metolachlor -19.00%	19.09	0.55	18.84	0.87	19.18	0.49

[a] Laboratory of FQSRI, PARC. [b] Laboratory of Syngenta Pakistan Limited. [c] Laboratory of PCSIR.

Table 6. **Robustness for simultaneous determination method of mesotrione, atrazine, benoxacor and S-metolachlor.**

Parameters	Variables	Mesotrione		Atrazine		Benoxacor		S-Metolachlor	
		N[a]	T[b]	N[a]	T[b]	N[a]	T[b]	N[a]	T[b]
Retention time, min	68-32	5119.84	0.86	4837.78	1.05	5258.92	1.01	5931.71	1.06
	70-30	8075.11	1.21	3767.23	1.01	4082.97	0.94	4982.76	0.98
	72-28	7434.69	1.47	3706.21	0.98	4363.27	0.95	5166.59	1.02
Wavelength, nm	258	5168.06	0.98	4325.90	0.98	4373.68	0.93	5263.29	1.01
	260	6711.63	1.20	4597.91	1.02	4590.25	0.94	4583.84	0.97
	262	6711.64	1.24	4143.20	0.99	4213.52	0.09	5210.06	1.00
Flow rate, mL/min	0.9	7973.47	1.31	3277.30	1.05	4227.39	0.93	5777.72	1.01
	1.0	8150.84	1.21	3618.50	1.01	3822.26	0.94	4886.46	0.98
	1.1	5960.15	1.19	3579.90	0.98	3654.43	0.94	4906.99	1.00

[a] Theoretical plate. [b] Tailing factor.

Robustness

For the robustness check of the proposed method, few pondered variations from the optimized conditions of developed method were taken into consideration. Since, the results were not found considerably changed by minor variation in wavelength, and composition or flow rate of mobile phase (Table 6), it proves the method flexibility and authenticity. Overall, the currently established method for quantification of all selected analytes in pesticide formulates is robust [13].

Conclusion

This study develops, validates and proposes a chromatography-based method for concomitant quantification of mesotrione, atrazine, benoxacor and S-metolachlor in pesticide formulates. The suggested method allows a good chromatographic peak separation and quantification with fair precision, accuracy and sensitivity. Inter-laboratory comparison provided Z-score values less than 2 for all formulation types. The reliability of the method was further confirmed by parameters such as robustness, linearity and system suitability test. Therefore, the developed method is effective, affordable, rapid, accurate and precise, and hence could be conveniently utilized for routine analytical tests in quality inspection labs.

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Conflict of Interest

The authors declare no conflicts of interest.

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