Development and validation of HPLC methods for the quantification of impending genotoxic impurities in Dapson drug substances

1. ABSTRACT:

The assessment of toxicological concentrations of possible genotoxic contaminants in drug substances was regarded as an important and challenging discipline . The International Conference and Harmonization (ICH) recommended that most pharmaceutical products be allowed to include 1.5 µg/day of a genotoxic contaminant. The goal study was to develop a quick and accurate HPLC method for measuring potential genotoxic impurities (PGIs) in Dapson drug substances.The chromatographic conditions were appropriately optimized to achieve a decent separation of each impurities peak with the Dapsone. A C8 column has been used with Phosphate buffer with the linear gradient combination with Acetonitrile as mobilephase and multipe wavelength used based on UV maxima of respective impurity. According to ICH criteria for the quantification of each impurity, method validation for HPLC was carried out regarding specificity, the limit of detection (LOD), the limit of quantification (LOQ), linearity, accuracy (recovery), precision, and solution stability. Three separate batches of Dapsone were successfully subjected to the desired procedures for the GTI determination and found not detected. The corelation coefficient observed > 0.99 in linearity and 70% to 130% recovery observed in the accuracy during method validation hence method can be considered linear and accurate and can be used for testing of genotoxic impurity in Dapsone drug substances..

Keywords: Genotoxic Impurities, HPLC analysis, Dapsone, Method development, Method validation, Dapson drug ,

2. INTRODUCTION:

Active pharmaceutical ingredients (APIs) are syntheses using a variety of chemical components, such as raw material, reactive intermediates, biological active material , catalysts etc. These substances may be present in the final product at a trace level. Based on their chemical makeup, characteristics, and reactivity, some of these substances were identified as probable genotoxic impurities (PGIs). These PGIs are unwelcome chemical substances that to alter genes, result in chromosomal breakage or rearrangements, and ultimately cause cancer [1]. To create safe pharmaceutical products, the measurement of these PGIs in API has received significant attention [2,3]. Guidelines for the control and analysis of these PGIs in drugs and drug products were released by regulatory bodies, including the European Directorate for the Quality of Medicines & Health Care (EDQM) in Europe and the Food and Drug Administration (FDA) in the United States [4,5].

GTIs have the potential to cause cancer in humans by causing genetic mutations, chromosomal rearrangements, or breaks [2]. As a result, even minimal exposure to these contaminants in API raises serious toxicological concerns [6]. Regulatory agencies have taken notice of the potential existence of GTIs in APIs [7,8]. Guidelines for GTI limits were released in 2006 by the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMEA) [6]. The term "Threshold of Toxicological Concern" (TTC) refers to a threshold exposure level to substances that do not significantly increase the risk of carcinogenicity or other toxic consequences [7,8,9]. GTI intake with a TTC value of 1.5 g/person/day is regarded as having a reasonable risk [7,8]. The ratio of TTC (g/day) and daily dose (g/day) determines the maximum concentration of GTI that can be present in a drug ingredient in ppm. The sensitivity criteria for the analytical method to be used are mostly determined by the concentration limit for a GTI [7,8]. By splitting GTIs into two groups based on their volatility, one can choose an analytical technique [2,7,8].

The target detection limit for the assessment of these PGIs in Dapsone drug substances should be less than 5 ppm in API, according to the published recommendations. The limit set for classical impurity analysis will be 200 times lower than this one [4,6]. Many conventional quality control techniques, which rely on the direct injection and separation of PGI-containing APIs, followed by HPLC-UV analysis, were ineffective for both qualitative and quantitative analyses of PGIs [10,11,12]. To detect and quantify minute organic compounds, adulterants in food and pharmaceutical items, including PGIs.

As depicted route of synthesis in [Figure 1 to Figure 3], Dapsone is manufactured in three steps are chemical conversation and one step is purification, The eleven impurities have been identified from the isomers of Key Starting Material (KSM), process impurities and intermediates and considered as potential genotoxic impurities based on Darek Sarah assessment [Table 2] and limit has been calculated based on ICH M7 guideline [Table 3]. Some of the impurities were identified with short names as described in Table 1,

Table 1. Short name of GTI impurities

Sr. No.	Chemical name of Impurity	Short Name of impurity
01	bis(4-nitrophenyl)sulfane	SON-I
02	4, 4'-sulfinylbis(nitrobenzene)	SON-II RC-I
03	4,4'-sulfonylbis(nitrobenzene)	SON-II
04	p-Chloro nitrobenzene	ZCL 18011
05	4-((4-Nitrophenyl)Thio)Aniline	SON-I RC-2
06	Bis (P-Nitrophenyl) Disulfied	SON-I RC-4

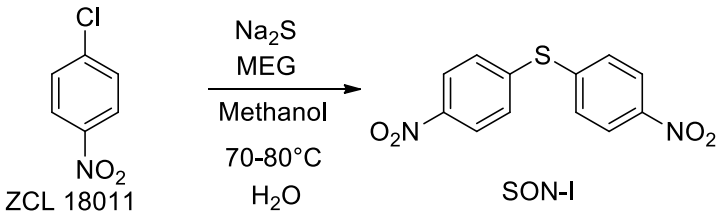


Figure 1. Synthesis scheme of Dapsone stage 1 (ZCL 18011 to SON-I)

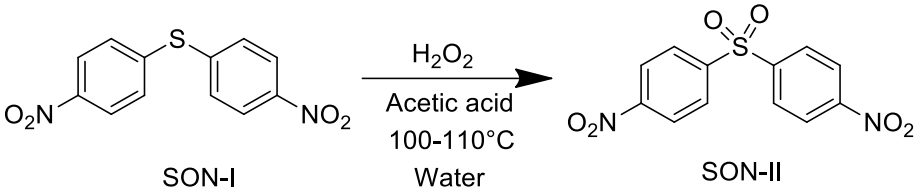


Figure 2. Synthesis scheme of Dapsone stage 2 (SON-I to SON-II)

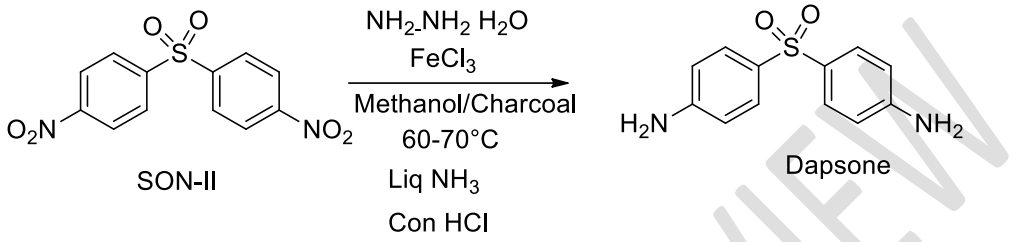
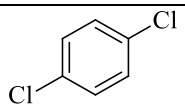
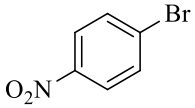


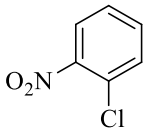
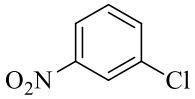
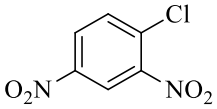
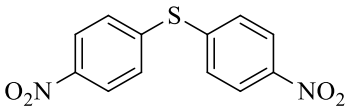
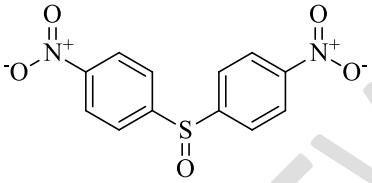
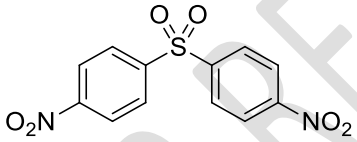
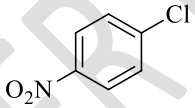
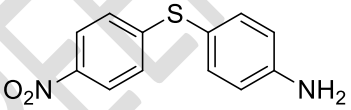
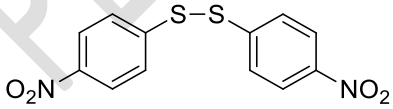
Figure 3. Synthesis scheme of Dapsone stage 3 (SON-II to Dapsone)

Table 2. Darek Sarah Assessment of all PGI Impurities of Dapsone

Sr. No.	Name of Product	Derek Prediction	Sarah Prediction	ICH M7 Class	Overall, in Silico	PGI Status
01	p-Dichlorobenzene	Inactive	Equivocal	Inconclusive	Inconclusive	Positive
02	1-Bromo-4-nitrobenzene	Plausible	Positive 100%	2	Positive	Positive
03	o-Chloro nitrobenzene	Plausible	Positive 100%	1	Positive	Positive
04	m-Chloro nitrobenzene	Plausible	Positive 30%	3	Positive	Positive
05	2,4-dinitro chlorobenzene	Plausible	Positive 100%	5	Positive	Positive
06	SON-I	Plausible	Positive 46%	3	Positive	Positive
07	SON-II RC-I	Plausible	Positive 39%	3	Positive	Positive
08	SON-II	Plausible	Positive 40%	3	Positive	Positive
09	ZCL 18011	Plausible	Positive 100%	1	Positive	Positive
10	SON-I RC-2	Plausible	Positive 100%	2	Positive	Positive
11	SON-I RC-4	Plausible	Positive 100%	2	Positive	Positive

Table 3. Limit of all eleven Genotoxic impurities (GTIs) in Dapsone API

Sr. No.	Name of Product	Structure	CAS No	Limit (Maximum)
01	p-Dichlorobenzene		106-46-7	5 ppm
02	1-Bromo-4-nitrobenzene		586-78-7	5 ppm

Sr. No.	Name of Product	Structure	CAS No	Limit (Maximum)
03	o-Chloro nitrobenzene		88-73-3	5 ppm
04	m-Chloro nitrobenzene		121-73-3	5 ppm
05	2,4-dinitro chlorobenzene		97-00-7	5 ppm
06	(SON-I)		1223-31-0	5 ppm
07	SON-II RC-I		119-59-5	5 ppm
08	SON-II		1156-50-9	5 ppm
09	ZCL 18011)		100-00-5	5 ppm
10	SON-I RC-2		101-59-7	5 ppm
11	SON-I RC-4		100-32-3	5 ppm

3. EXPERIMENT:

3.1 Chemicals, reagents and standards:

Dapsone drug manufacture by Cohance lifesciences Ltd, Ankleshwar (EP/USP Grade, HPLC purity > 99.9%), Impurity standards of SON-II RC-1 (HPLC purity 88.83%). SON-II (Potency 96.79%), SON-I RC-2 (Potency 99.78%), SON-I (Potency 98.98%), SON-I RC 4 (HPLC purity 76.53%) were used from Cohance Lifesciences Ltd. (Ankleshwar Gujarat). Impurity standard 2,4 Dinitro Chlorobenzene (GC purity 99.61%) were purchased from Avra Synthesis Pvt Ltd, Hyderabad. Impurity standards O-Chloronitrobenzene (GC purity 99.96%), M-Chloronitrobenzene (GC purity 99.1%), 1,4-Dichlorobenzene (GC purity 99.95%) were purchased from Loba Chemie Pvt. Ltd. (Tarapur Boisar Palghar). Impurity standard 1-Bromo-4-nitrobenzene (GC purity 99.86%) was purchased from Spectrochem Pvt. Ltd. (Tarapur Boisar Palghar). Orthophosphoric acid, 85% AR grade used from Spectrochem and Merck, Acetonitrile used from SD fine Chemical Ltd. Purify Water used from Milli Q (Merck)

3.2 Chromatographic Condition :

HPLC-DAC system from Waters Corporation (Model Alliance 2998 USA) and Shimadzu (Model LC-2010 CHT Japan) with a quaternary pump, and an automated injector (15°C temperature) was used. and a thermostat column. Shim-pack GIST C8 (250 mm x 4.6 mm x 3 µm) (Shimadzu Japan) HPLC

column used with the 55°C column oven temperature. The mobile phase was composed of phosphate buffer and Acetonitrile, in a gradient condition with an initial ratio of buffer and Acetonitrile (75:25) to (40:60) with the 1.4 ml/min flow rate. A 50 µL Injection volume was made. Multiple UV detection was set. at 258 nm, 321 nm and 222 nm. The entire analysis took 65 minutes of run time.

Standard and sample preparation:

10 mg of each impurity 2,4-Dinitro chlorobenzene, O-Chloro nitrobenzene, P-Chloro nitrobenzene, M-Chloro nitrobenzene, 1-Bromo-4-nitrobenzene, SON-1 RC-2, SON-I, SON-I RC-4 and 1,4-Dichlorobenzene standard was diluted with 100 mL of Acetonitrile and made 100 mg/L stock solution, 10 mg of each impurity SON-II RC-1 and SON-II standard was diluted with Acidic Acetonitrile (0.5% ortho-phosphoric acid (85%) in Acetonitrile) and made 100 mg/L stock solution, 0.1 ppm final solution made diluted with diluent (Mixture of Buffer and acetonitrile (40:60)).

In the case of the Dapsone API 20,000 ppm ppm sample was prepared in the mobile phase. All samples and standards were filtered through 0.22mm PVDF filter paper (Merck, Durapore PVDF 0.22, PL 47mm) before injection.

3.3 Method validation:

The method validation was performed as per ICH Q2 (R1) with relation to specificity, Limit of Detection (LOD), Limit of Quantification (LOQ), Linearity, Accuracy (Recovery), solution stability and followed by Batch analysis. In particular specificity was demonstrated by spike samples containing the target GTIs, coupled with blanks and sample which do not contain these analytes. In agreement with ICH guidelines, representative chromatograms were selected to demonstrate specificity and individual components were appropriately labelled. Furthermore, peak purity tests were carried out by HPLC-DAD to show that the analyte chromatographic peak was not attributable to more than one component. Regarding LOD and LOQ, it was evaluated considering the analyte concentration that would yield a signal-to-noise (S/N) value of more than 3 and 10 respectively. The LOD and LOQ values were experimentally verified by injections of each impurity standard solution of the compounds at the respective LOD/LOQ concentrations.

Regarding specificity, limit of detection (LOD), limit of quantification (LOQ), linearity, precision, accuracy (recovery) and solution stability, the technique was validated the following as per ICH Q2 (R1) [13,14]. Spike samples containing the target GTIs, together with blanks and samples which do not include these analytes, were used to establish specificity, which was then followed by batch analysis in particular. The following ICH recommendations, representative chromatograms were chosen to show specificity and individual components were labelled correctly. Additionally, HPLC-DAD performed peak purity tests to demonstrate that the analyte chromatographic peak could not be attributed to more than one component. The analyte concentration that would provide a signal-to-noise (S/N) value of more than 3 and 10, respectively, was taken into consideration while evaluating LOD and LOQ. Linearity was performed with the range of LOQ to 150% of specification level with the five levels of interval and correlation coefficient (r) was evaluated for each GTI. Precision was evaluated with the six preparations of spike samples containing the target GTIs and results were evaluated with the Relative standard deviation. Accuracy was evaluated in terms of recovery by adding a known amount of each impurity at LOQ, specification level and 150% of the target concentration of each GTI and results have been evaluated within the recovery limit between 70.0% to 130%. Solution stability has been evaluated with the spike samples containing the target GTIs with different time intervals and a solution was found stable up to 24 Hrs at about 15C temperature.

4. RESULTS AND DISCUSSION:

The objective of this work was to develop an easy-to-use, sensitive HPLC method with a multiple wavelength approach for the detection and measurement of eleven PGIs in the Dapsone drug substances. The method's ability to resolve and detect all eleven PGIs at trace levels along with conventional Dapsone was taken into consideration when it was first optimized. For resolving eleven PGI impurities, various C8 and C18 column designs with various column temperatures were investigated. various solvent compositions Phosphoric acid was mixed with other substances, including acetonitrile and methanol. The stationary phase used in the resolution of the analytes was Shim-pack GIST C8 (250 mm x 4.6 mm x 3 µm), and the optimal conditions for the resolution of PGIs in Dapsone were determined to be acetonitrile and 0.1% v/v Phosphoric acid with linear gradient at 1.4 mL/min flow rate. The multiple Ultraviolet wavelengths were evaluated on a Photodiode array (PDA) detector to get the proper response of respective PGI impurities. The 258 nm, 321 nm and 222 nm wavelengths were used to determine respective impurities.

The standard solution of all impurities was spiked into the sample at the specification level (5 ppm) by analyzing 20 mg/mL concentrations of Dapsone and its PGIs and in combination with a blank, the specificity of the approach was confirmed. Figures 4, 5 & 6 show the chromatograms that were observed for the spike analysis at 258nm, 321nm and 222 nm overlapping with blank chromatograms; the chromatogram doesn't show any chromatographic reactions during the whole run. analysis period. The fact that there was no extra detection of unwanted chemicals or contaminants in the study's spike chromatogram, which separates the analytes, indicates that the method was designed specifically for the analysis of PGIs in Dapsone.

The sample and its PGIs were individually analyzed, as was seen in the optimized approach, and the retention time (tR) was compared to combined solution analysis. The individual analysis's Retention time (RT) of all PGIs mentioned in Table 4. The individual analytes' analyses were compared based on the RT of the detected analytes. in conjunction with the RT of peaks found in the combined solution analysis. The selectivity of the established approach was confirmed by the observation that the RT of the analytes' individual analyses was identical to the peaks found for combined analyses. The method specificity for the analysis of all PGIs is confirmed by the absence of additional compounds or contaminants in the run time of combined standard, individual standard solutions, as well as the blank analysis.

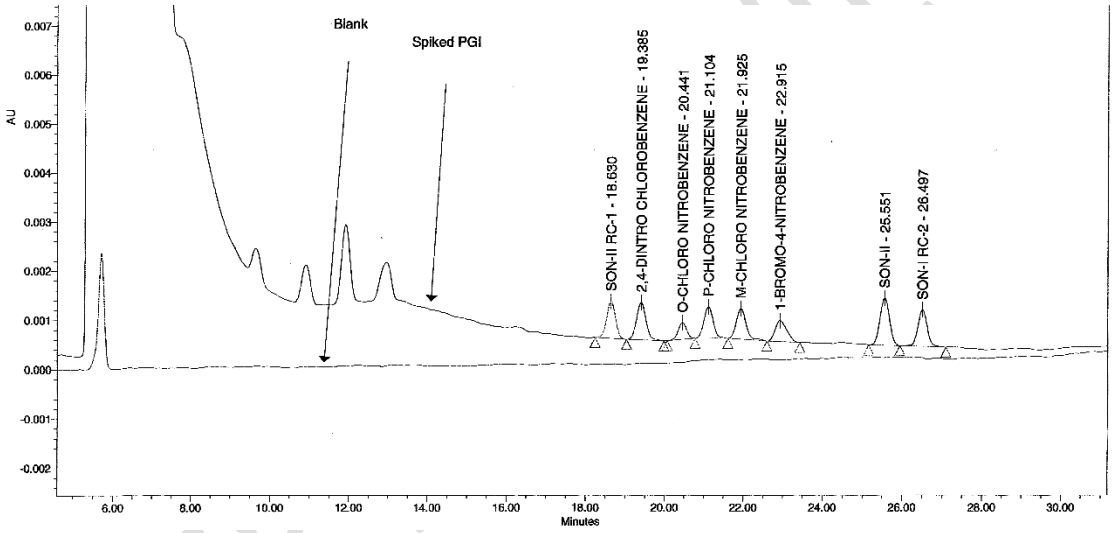


Figure 4. Spike chromatograms at 258 nm wavelengths (Eight PGIs)

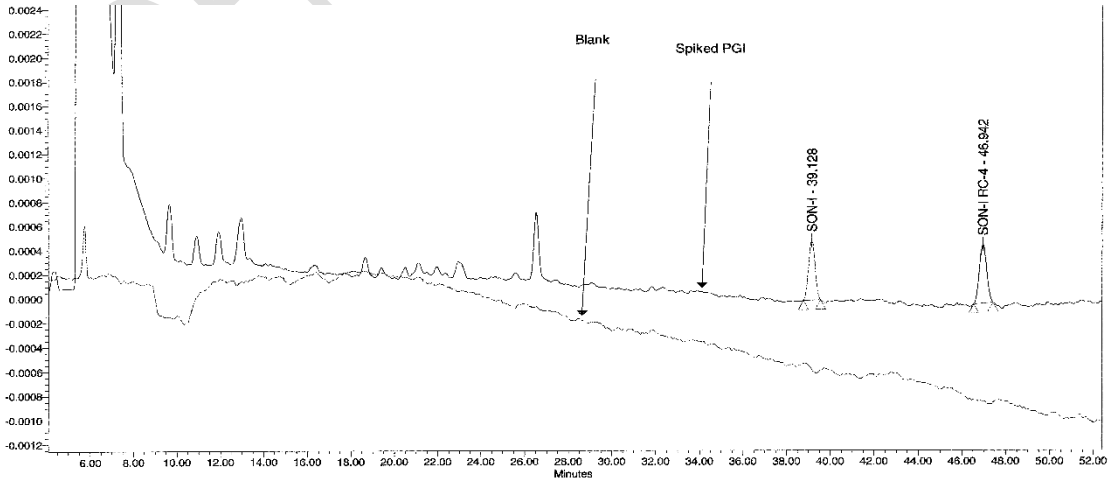


Figure 5. Spike chromatograms at 321 nm wavelengths (Two PGIs)

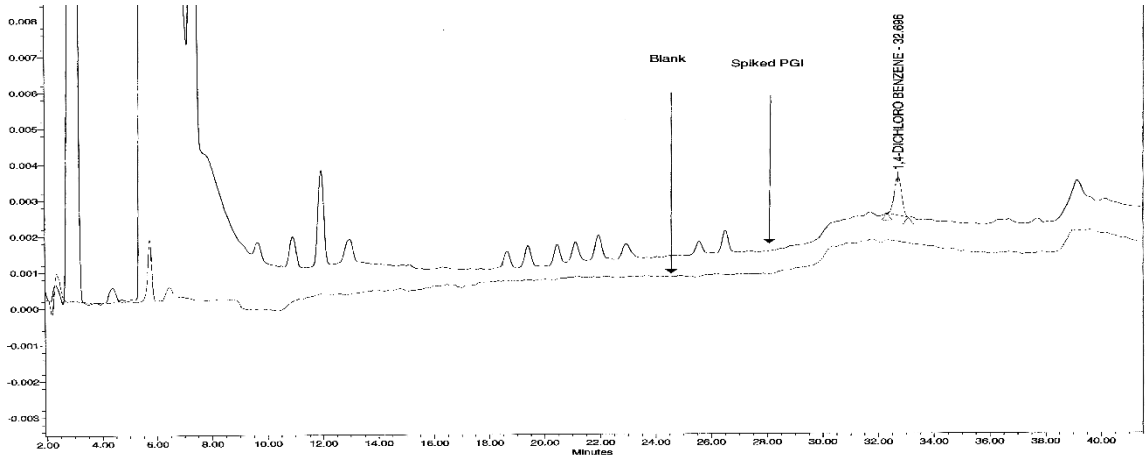


Figure 6. Spike chromatograms at 222 nm wavelengths (One PGI)

Table 4. Retention timetable & UV wavelength of each PGI

Sr. No.	Name of impurity	Retention time (min)	Wavelength(nm)
1.	SON-II RC-1	18.3	258 nm
2.	2,4-Dinitro Chlorobenzene	18.9	258 nm
3.	O-Chloro nitrobenzene	20.1	258 nm
4.	P-Chloro nitrobenzene	20.8	258 nm
5.	M-Chloro nitrobenzene	21.7	258 nm
6.	1-Bromo-4-nitrobenzene	22.8	258 nm
7.	SON-II	25.8	258 nm
8.	SON-I RC-2	26.9	258 nm
9.	SON-I	40.6	321 nm
10.	SON-I RC-4	48.2	321 nm
11.	1,4-Dichlorobenzene	34.9	222 nm

4.1 Method validation:

All eleven PGIs were analyzed at varied concentrations to establish the linearity range of the analytes. The calibration curve was created by plotting the individual analyte's area response on the y-axis and its strength on the x-axis. By using high correlation concentration ranges for the study's analytes, the calibration range was confirmed. For all eleven PGIs, the accurate fit with a high correlation calibration curve was seen in the range of 1.5 µg/mL to 7.5 µg/mL.

To assess calibration curve parameters such the Correlation Co-efficient (r), intercept, and Residual sum of squares, As shown in Table 5, the regression line and calibration equation for all eleven PGIs shown in Figure 7 to Figure 17.

Table 5.Correlation Co-efficient (r), intercept, and Residual sum of squares of each PGI

Sr. No.	Name	Correlation Co-efficient (r)	y-Intercept	Residual sum of squares
1.	SON-II RC-1	0.9948	-258.1686	585.4036

Sr. No.	Name	Correlation Co-efficient (r)	y-Intercept	Residual sum of squares
2.	2,4-Dinitro Chlorobenzene	0.9963	1020.8229	472.0282
3.	O-Chloro nitrobenzene	0.9922	1441.6585	379.6186
4.	ZCL 18011	0.9960	836.8314	414.5924
5.	M-Chloro nitrobenzene	0.9977	-355.6071	364.2682
6.	1-Bromo-4-nitrobenzene	0.9972	295.5616	308.2338
7.	SON-II	0.9930	1850.9831	861.2248
8.	SON-I RC-2	0.9977	593.7858	368.5198
9.	SON-I	0.9950	129.4789	501.9857
10.	SON-I RC-4	0.9914	365.8263	751.0933
11	1,4-Dichlorobenzene	0.9975	-115.4992	712.0678

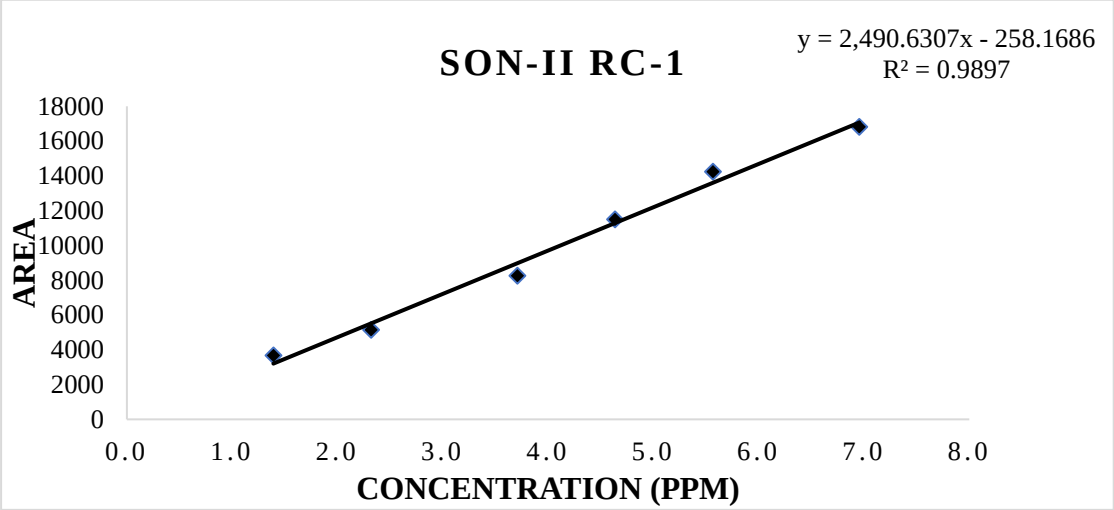


Figure 7. Regression line and calibration equation of SON-II RC-1

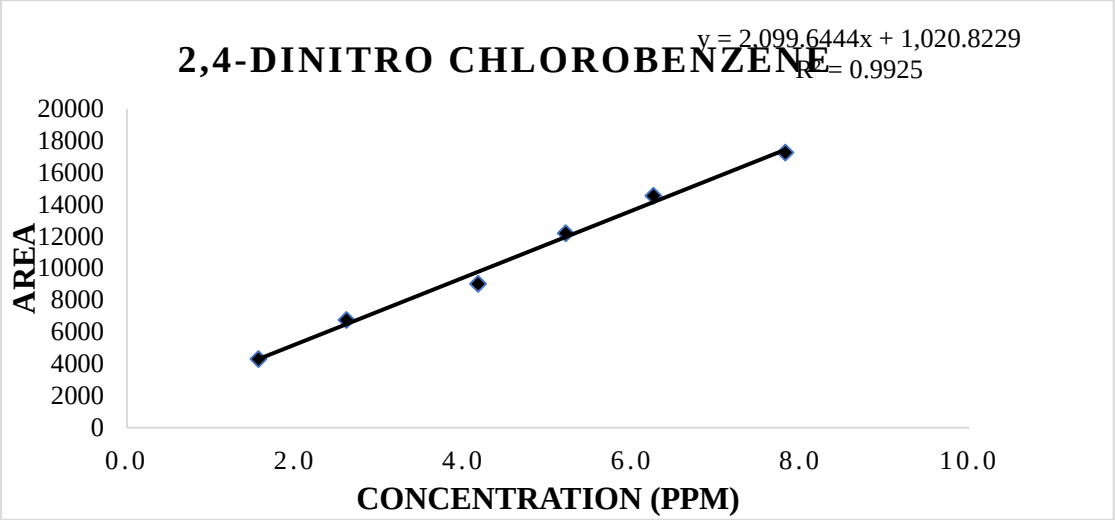


Figure 8.Regression line and calibration equation of 2,4-Dinitro Chlorobenzene:

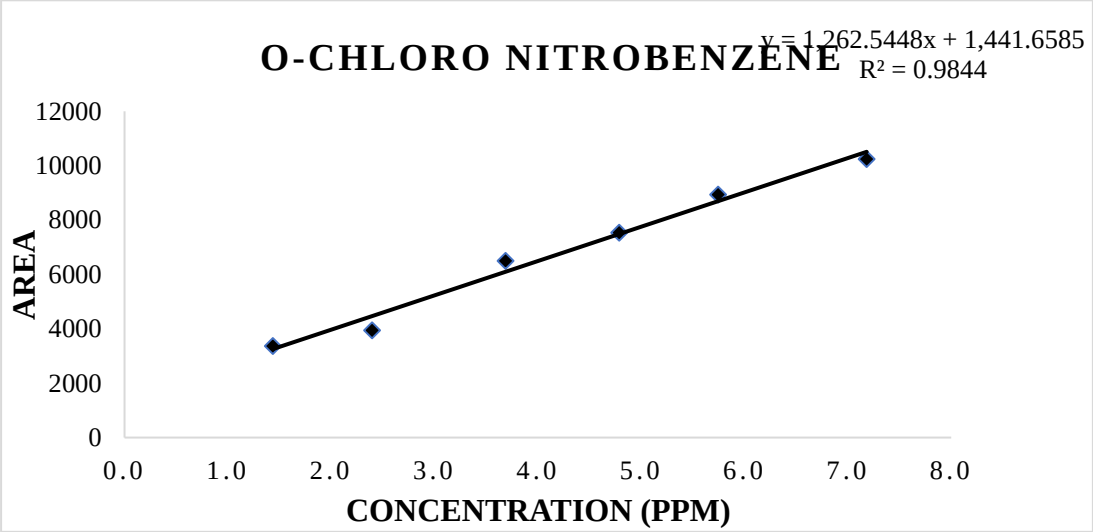


Figure 9. Regression line and calibration equation of O-Chloro nitrobenzene

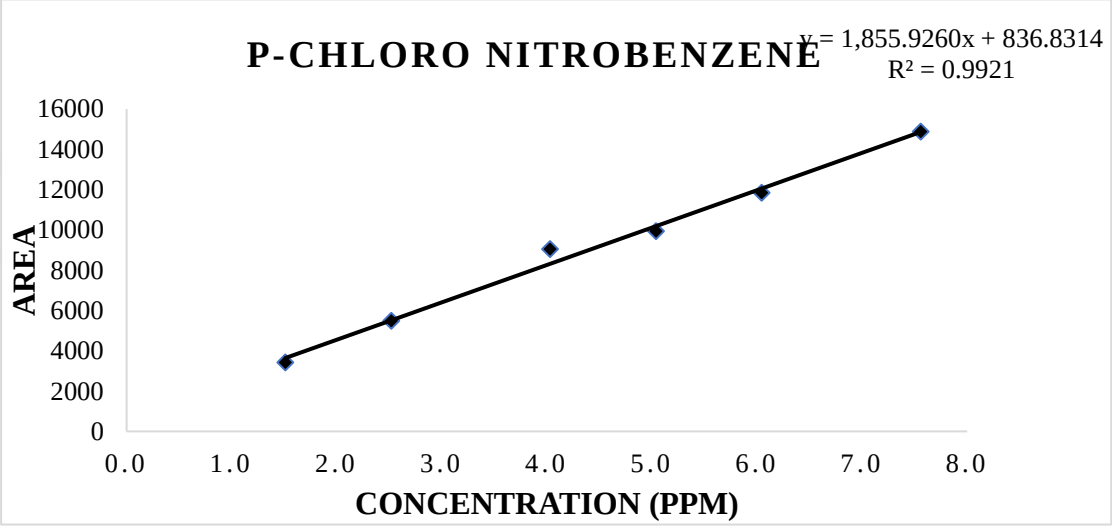


Figure 10. Regression line and calibration equation of P-Chloro nitrobenzene

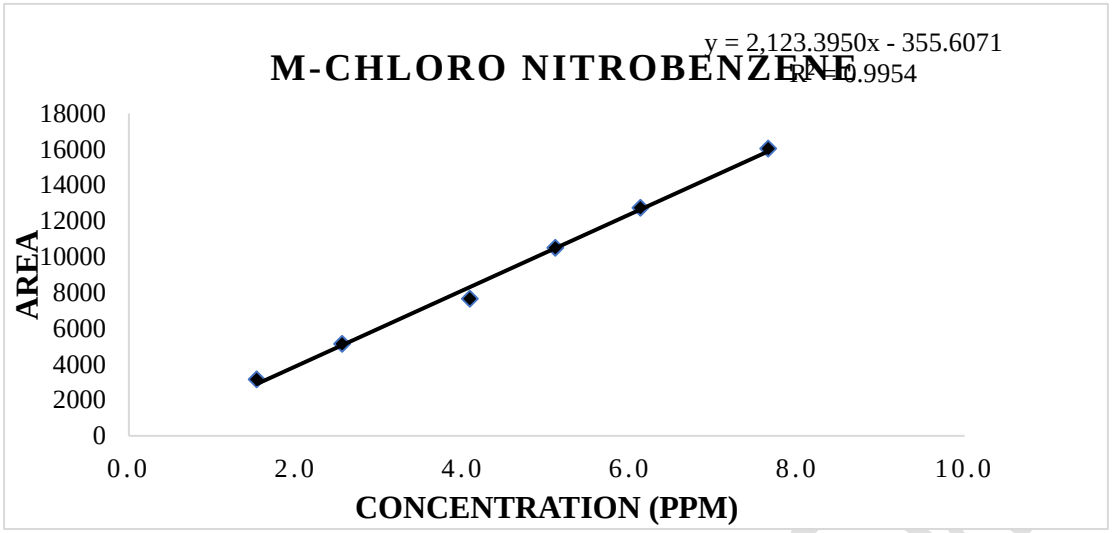


Figure 11 Regression line and calibration equation of M-Chloro nitrobenzene:

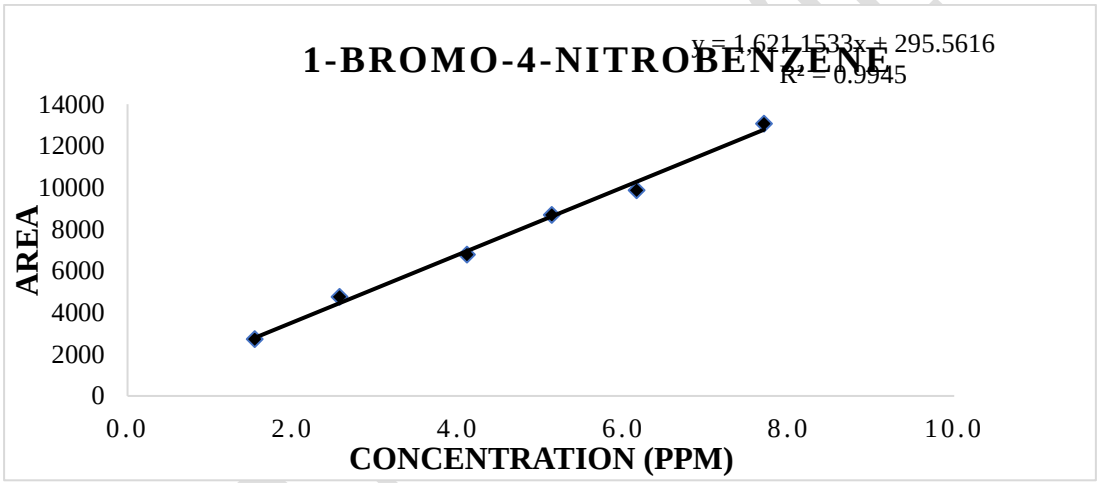


Figure 12 Regression line and calibration equation of 1-Bromo-4-nitrobenzene:

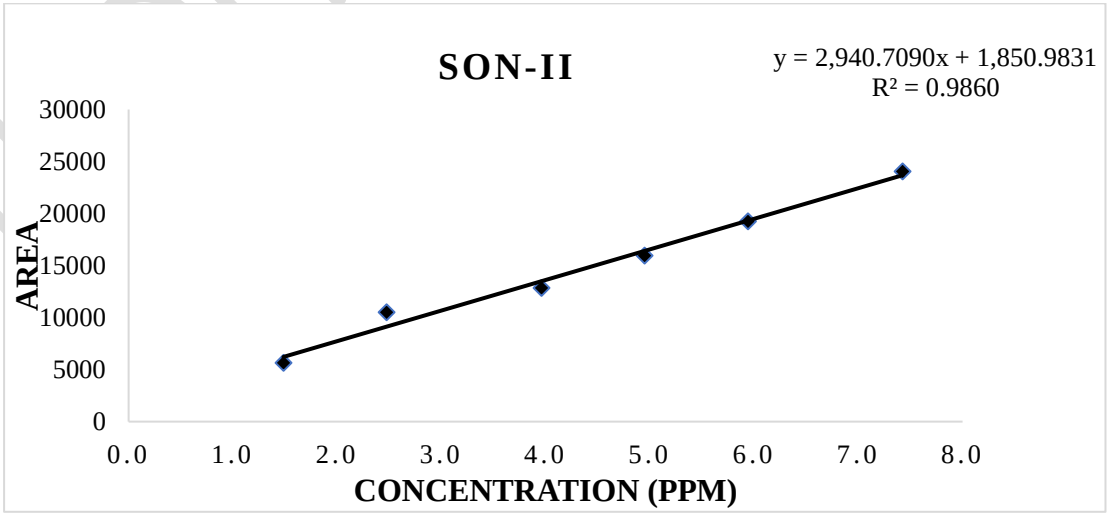


Figure 13 Regression line and calibration equation of SON-II:

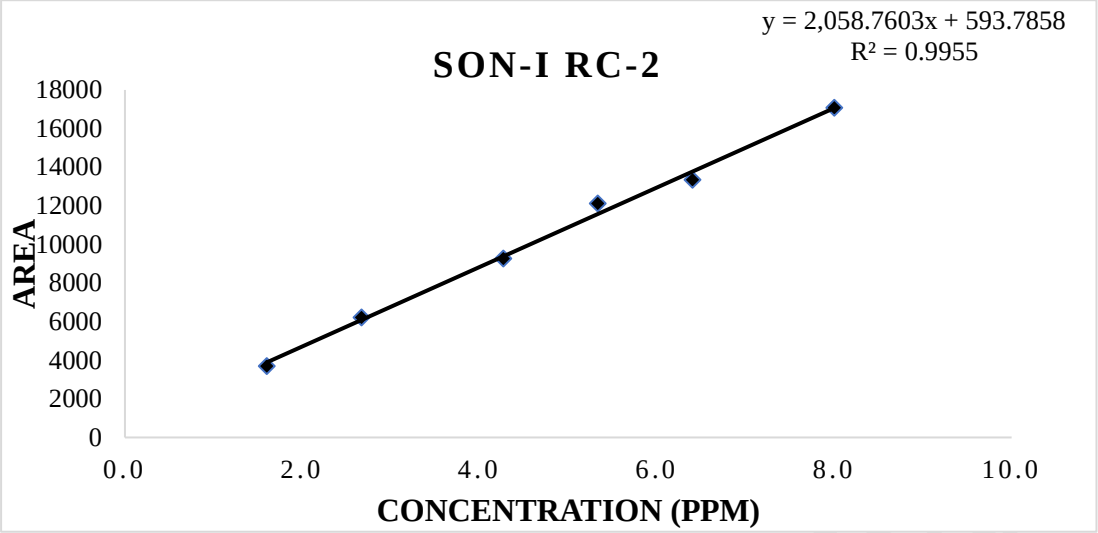


Figure 14 Regression line and calibration equation of SON-I RC-2:

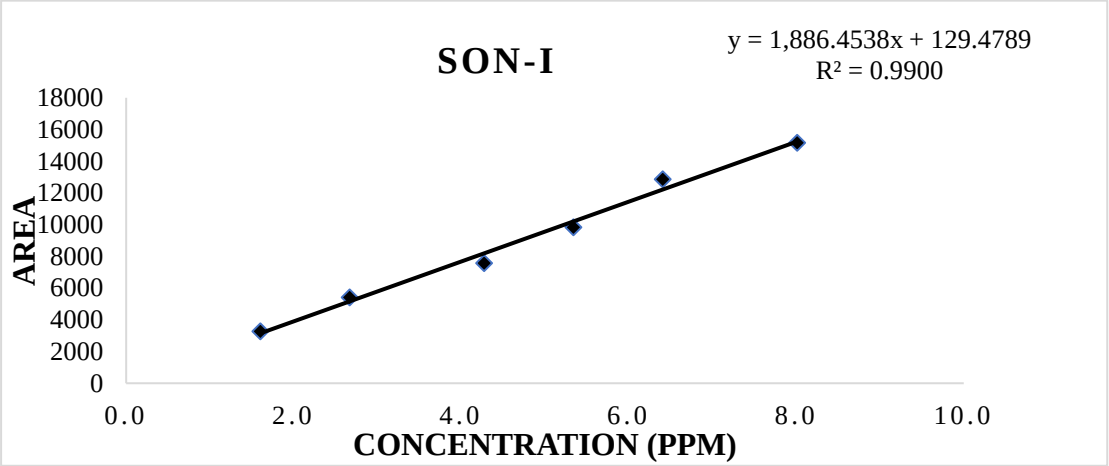


Figure 15 Regression line and calibration equation of SON-I :

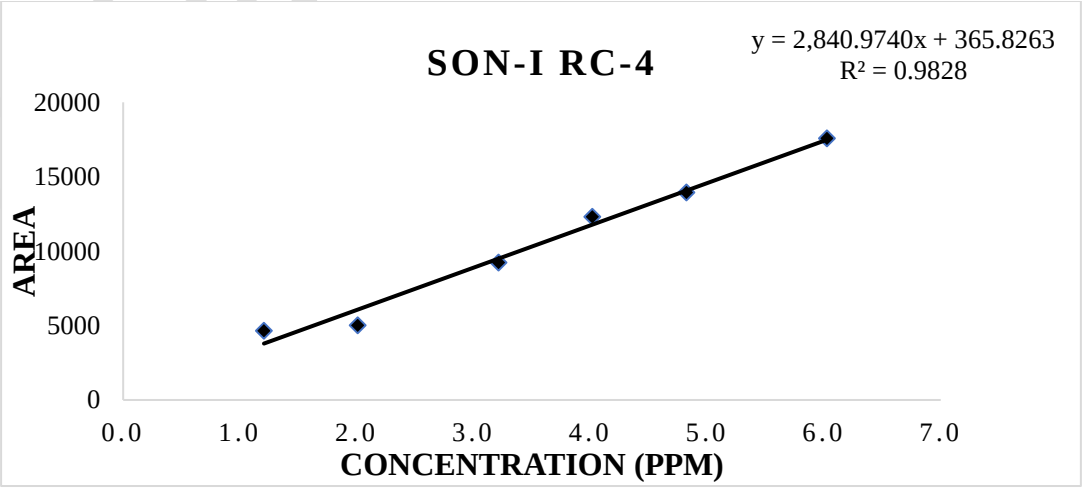


Figure 16 Regression line and calibration equation of SON-I RC-4 :

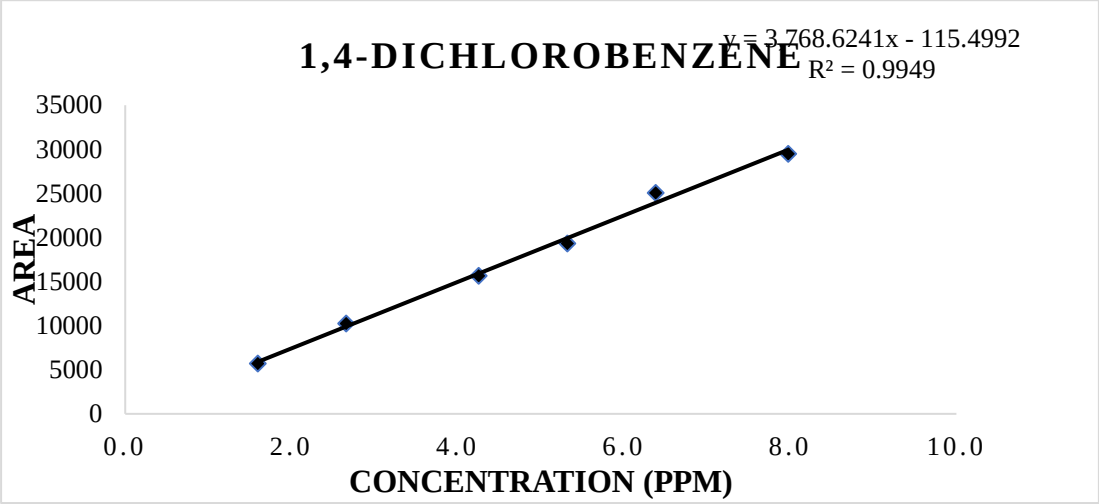


Figure 17 Regression line and calibration equation of 1,4-Dichlorobenzene:

The recovery experiment was carried out in the study at the linearity level for all eleven PGIs at concentrations of 1.5 µg/mL (LOQ level), 5.0 µg/mL (100% of Specification level), and 7.5 µg/mL (150% of specification level). The area results of each analyte were compared with its corresponding peak area response on the calibration level, and the recovery level concentration solution was made and analyzed in triplicate using the devised method. The percentage recovery of each analyte and percentage relative standard deviation (% RSD) at the studied spiked level were computed at Table 6.

Tabel 6. Percent recovery and percent relative standard deviation (% RSD) of each PGI

Peak Name	Set No.	LOQ (Level-1) (1.5 µg/mL)	100% (Level-2) (5.0 µg/mL)	150% (Level-3) (7.5 µg/mL)
SON-II RC-1	1	99.13	112.22	98.25
	2	92.05	99.06	96.69
	3	86.01	102.54	100.08
	%Mean recovery	92.40	104.61	98.34
	%RSD	7.11	6.52	1.73
2,4-Dinitro Chloro benzene	1	101.66	90.90	102.23
	2	101.49	97.38	102.96
	3	97.87	101.88	103.41
	%Mean recovery	100.34	96.72	102.87
	%RSD	2.13	5.70	0.58
ZCL 18011	1	90.34	92.27	95.32
	2	91.41	94.21	98.34
	3	95.90	95.09	93.91
	%Mean recovery	92.55	93.86	95.86

Peak Name	Set No.	LOQ (Level-1) (1.5 µg/mL)	100% (Level-2) (5.0 µg/mL)	150% (Level-3) (7.5 µg/mL)
	%RSD	3.19	1.54	2.36
	1	97.30	86.27	100.58
	2	91.71	92.40	98.18
M-Chloro nitrobenzene	3	114.30	97.41	99.56
	%Mean recovery	101.10	92.03	99.44
	%RSD	11.64	6.06	1.21
	1	120.45	87.66	100.80
	2	98.92	89.09	100.33
1-Bromo-4-nitrobenzene	3	82.09	86.89	101.15
	%Mean recovery	100.49	87.88	100.76
	%RSD	19.14	1.27	0.41
	1	98.63	96.37	100.36
	2	110.98	99.97	100.51
SON-II	3	112.15	106.50	102.11
	%Mean recovery	107.25	100.95	100.99
	%RSD	6.99	5.09	0.96
	1	88.50	102.56	100.18
	2	99.54	109.33	100.46
	3	93.11	114.00	102.15
SON-I RC-2	%Mean recovery	93.72	108.63	100.93
	%RSD	5.92	5.30	1.06
	1	122.87	96.54	94.28
	2	126.02	95.77	96.60
1,4-Dichloro	3	127.68	100.14	99.93
benzene	%Mean recovery	125.52	97.48	96.94
	%RSD	1.95	2.39	2.93
	1	93.07	90.18	96.77
SON-I	2	94.73	94.95	96.38
	3	107.62	93.92	99.31

Peak Name	Set No.	LOQ (Level-1) (1.5 µg/mL)	100% (Level-2) (5.0 µg/mL)	150% (Level-3) (7.5 µg/mL)
SON-I RC-4	%Mean recovery	98.47	93.01	97.49
	%RSD	8.09	2.70	1.63
	1	70.07	98.44	100.18
	2	94.44	98.73	103.57
	3	105.94	92.19	100.92
	%Mean recovery	90.15	96.46	101.56
	%RSD	20.32	3.83	1.75

The percentage recovery for all eleven PGIs is between 80.0% to 120% at 100% & 150% level and between 70.0% to 130% at LOQ level. The percentage RSD for each studied level of the analytes was calculated, and the findings were found to be less than 10.0% at 100% & 150% levels and less than 25% at the LOQ level, which was acceptable by the standards. The fact that the findings were below the permitted level indicates that the approach was accurate and recoverable. Table 6 shows the outcomes of the recovery study using the technique that was chosen to analyze Dapsone for its PGIs.

Table 7: percent relative standard deviation (% RSD) of method repeatability of each PGI

Sr. No.	Name	% RSD
1	SON-II RC-1	5.80
2	2,4-Dinitro Chlorobenzene	4.35
3	O-Chloro nitrobenzene	5.38
4	ZCL 18011	3.30
5	M-Chloro nitrobenzene	4.74
6	1-Bromo-4-nitrobenzene	4.16
7	SON-II	3.53
8	SON-I RC-2	3.46
9	SON-I	3.02
10	SON-I RC-4	3.95
11	1,4-Dichlorobenzene	2.17

All eleven PGIs at a concentration of 5 µg/mL were analyzed to determine the method's repeatability and reproducibility. Six injections were made into the solution in a single day for intraday precision. The peak response of all eleven PGIs were compiled, According to the criteria, a % RSD of less than 10 was regarded as acceptable, and based on the results presented in Table 7, the fact that the results were obtained were below the acceptable threshold shows the accuracy and reproducibility of the procedure.

LOD (limit of detection) and LOQ (limit of quantification) were used to express the sensitivity of all eleven PGIs in the established approach. The signal-to-noise ratio technique was used to assess the

LOD and LOQ of each PGI. The s/n ratio of 3 was regarded as LOD and the s/n ratio of 10 as LOQ. The LOD and LOQ values of all eleven PGIs are mentioned in Tables 8 and 9. The LOQ value was found less than 30% of the TTC value, which is acceptable as per ICH M7. The results of the sensitivity test show that the proposed method can detect all PGIs at a very low concentration of 0.01 µg/mL (ppm) as such concentration.,

Table 8: signal-to-noise ratio (S/N) ratio of each PGI at the limit of detection (LOD) level

Sr. No.	Name	Conc. as such in ppm	Conc. wrt test in ppm	Area	S/N
1	SON-II RC-1	0.009	0.5	2017	15
2	2,4-Dinitro Chlorobenzene	0.010	0.5	1540	14
3	O-Chloro nitrobenzene	0.010	0.5	1534	10
4	ZCL 18011	0.010	0.5	1385	12
5	M-Chloro nitrobenzene	0.010	0.5	1643	12
6	1-Bromo-4-nitrobenzene	0.010	0.5	1238	8
7	SON-II	0.010	0.5	2445	19
8	SON-I RC-2	0.011	0.5	1392	12
9	SON-I	0.011	0.5	780	5
10	SON-I RC-4	0.008	0.4	1400	9
11	1,4-Dichlorobenzene	0.011	0.5	2745	19

Table 9: signal-to-noise ratio (S/N) ratio of each PGI at limit of detection (LOD) level

Sr. No.	Name	Conc. (ppm) as such	(ppm) Conc. wrt test	Area	S/N
1.	SON-II RC-1	0.028	1.4	3765	22
2.	2,4-Dinitro Chlorobenzene	0.031	1.6	3122	18
3.	O-Chloro nitrobenzene	0.029	1.4	1945	11
4.	ZCL 18011	0.030	1.5	3102	16
5.	M-Chloro nitrobenzene	0.031	1.5	4249	20
6.	1-Bromo-4-nitrobenzene	0.031	1.5	2346	11
7.	SON-II	0.030	1.5	4942	29
8.	SON-I RC-2	0.032	1.6	4106	22
9.	SON-I	0.032	1.6	3223	19
10.	SON-I RC-4	0.024	1.2	3002	16
11.	1,4-Dichlorobenzene	0.032	1.6	6972	36

By allowing the spike solution with all eleven PGI impurities at TTC level (5 ppm) to incubate in an auto-sampler for 24 hours at 15°C, it was possible to gauge the stability of the solution created for the analysis of all eleven PGIs. The incubated solution was examined using the proposed methodology for 18 Hrs. 24 Hrs and 30 Hrs. By comparing the area findings of each analyte with its matching with the initial analysis, the area ratio of each station was calculated. For analytes up to 24 hours, the area ratio was seen between 0.90 to 1.10 up to 24 hours and observed lower/higher side up to 30 Hrs. which data tabulated in table 10. This demonstrated that the produced solutions were stable for up to 24 hours at 15°C.

Table 10 Area ratio for solution stability of each PGI

Name	Spiked sample solution			
	Area ratio			
	Initial	18hr	24hr	30hr
SON-II RC-1	NA	0.99	1.05	0.98
2,4-Dinitro Chlorobenezene	NA	0.97	0.99	0.92
O-Chloro nitrobenzene	NA	0.95	1.08	1.20
ZCL 18011	NA	1.06	1.10	1.11
M-Chloro nitrobenzene	NA	1.00	1.07	0.97
1-Bromo-4-nitrobenzene	NA	0.99	1.09	1.12
SON-II	NA	1.01	1.04	1.10
SON-I RC-2	NA	1.01	0.99	1.04
SON-I	NA	0.99	1.03	1.04
SON-I RC-4	NA	1.07	1.04	1.07
1,4-Dichlorobenzene	NA	1.04	1.06	1.01

4

5 CONCLUSION:

To identify and quantify eleven PGIs, in Dapsone drug substances, a straightforward and sensitive analytical HPLC method approach was created and successfully verified in this work. Multiple wavelengths were used to detect the impurity at the respective wavelengths of each impurity into DAD detector, demonstrating that the analytical conditions were favourable. In the concentration range of range of 1.5 µg/mL to 7.5 µg/mL the method exhibits a linear calibration curve and the desired recovery of each impurity is isprecise, accurate, and specific. The method's sensitivity was demonstrated by the detection values, which were observed to be 0.5 µg/mL and 1.5 µg/mL LOQ value for all impurities. The technique can locate and measure PGIs in dose and pure drug forms. The technique can also be used for in-process impurity detection while Dapsone is being synthesized. based on the results obtained, it can be said that the study will guarantee the safe usage of Dapsone during production.

6 ACKNOWLEDGEMENT

I am thankful to Institute of Science and technology for advanced studies and research (ISTAR) and Charutar Vidya Mandal (CVM) university for providing infrastructure and facilities.

Also, I am grateful to Cohance lifesciences Ltd, Ankleshwar to provide infrastructure for the research software and financial support. Below associates were help to us for the providing technical support for the synthesized the impurities and provided analytical supports.

7 REFERENCES:

1) Europeanperspective. Toxicol Lett. **2004; 151(1):29-41.** https://doi.org/10.1016/j.toxlet.2004.04.
2) David F, Jacq K, Sandra P, Baker A and Klee MS. Analysis of potential genotoxic impurities in pharmaceuticals by two-dimensional gas chromatography with Deans switching and independent column temperature control using a low-thermal-mass oven module. Anal Bioanal Chem. **2010; 396(3):1291-300.** https://doi.org/10.1007/s00216-009-3304-9
3) Dobo KL, Greene N, Cyr MO, Caron S and Ku WW. The application of structure-based assessment to support safety and chemistry diligence to manage genotoxic impurities in active pharmaceutical ingredients during drug development. Regul Toxicol Pharmacol. 2006; 44(3):282-93 https:// doi. org/ 10.1016/j.yrtph.**2006.01.004**
4) Bartos Dorottya and Gorog, Sandor, Recent Advances in the Impurity Profiling of Drugs, Current Pharmaceutical Analysis, **2008, 4(4): 215-230** https://doi.org/10.2174/157 341 208 786306199
5) European Medicines Evaluation Agency, Committee for Medicinal Products for Human Use, Guideline on the limits of genotoxic impurities, CPMP/SWP/5199/02, **London, 28 June 2006**
6) Guidance for Industry. Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches. US Department of Health and Human Services, FDA, Center for Drug Evaluation and Research (CDER), **December 2008**
7) Ed SK and David SH. Emerging Targeted Therapy for Tumors with NTRK Fusion Proteins. Clin Cancer Res. **2018; 24(23): 5807–5814** https://doi.org/10.1158/1078-0432.CCR-18-1156
8) Cocco E, Scaltriti M and Drilon A. NTRK fusion-positive cancers and TRK inhibitor therapy. Nat Rev Clin **Oncol. 2018;** 15(12):731-747 https://doi.org/10.1038/s41571-018-0113-0
9) David SH, Steven GD, Shivaani K, Anna FF, et al. Larotrectinib in patients with TRK fusion-positive solid tumours: a pooled analysis of three phase 1/2 clinical trials. The Lancet **Oncology. 2020; 21(4): 531-540** https:// doi.org/10.1016/S1470-2045(19)30856-3
10) Mahreen Fatima, Anupama Koneru, M. Mushraff Ali Khan, Murali Balaram Varanasi and Imam Pasha Syed. Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Larotrectinib in its Formulations. **Orient J Chem. 2020;** 36(2): 327-333 http://dx.doi.org/10.13005/ojc/360216
11) Tripathy HK, Nair Manju SV, Zakkula A, Bestha RM, Dittakavi S and Mullangi R. Validated HPLC Method for Quantification of a Novel Trk Inhibitor, Larotrectinib in Mice Plasma: Application to a Pharmacokinetic Study. **Drug Res (Stuttg). 2020;** 70(2-03):101-106 https://doi.org/10.1055/a-1071-0849
12) DasameswaraRao K, Arthanareeswari M and Tamilselvi M. Identification and characterization of unknown degradation product of larotrectinib sulphate. Materials Today: **Proceedings. 2021;** 40(1): 172-179 https://doi.org/10.1016/j.matpr.2020.06.526
13) International council for harmonisation of technical requirements for pharmaceuticals for human use, ICH harmonised guideline, analytical procedure **development, Q14, 2022**
14) nternational Council for Harmonisation of technical requirements for pharmaceuticals for human use, ICH harmonised guideline, validation of analytical **procedures, Q2(R2), 2022**
15) Kumar PS, Rambabu K. Development and Validation of a Method for Simultaneous Estimation of Bupropion and Dextromethorphan Using Reverse Phase High Performance Liquid Chromatography in Active Pharmaceutical Ingredient Form. J. Pharm. Res. Int. [Internet]. 2022 Oct. 31 [cited 2024 May 21];34(52B):10-9. Available from: https://journaljpri.com/index.php/JPRI/article/view/7217
16) Wajngarten D, Pazos JM, Neves T da C, Garcia PPNS. Development and Validation of Class I Preparation and Restoration Quality Assessment Methods. J. Adv. Med. Med. Res. [Internet]. 2021 May 11 [cited 2024 May 21];33(11):18-23. Available from: https://www.journaljammr.com/index.php/JAMMR/article/view/4050
17) Subramanian VB, Katari NK, Dongala T, Jonnalagadda SB. Stability-indicating RP-HPLC method development and validation for determination of nine impurities in apixaban tablet dosage forms. Robustness study by quality by design approach. Biomedical Chromatography. 2020 Jan;34(1):e4719.