IJAPB International Journal of Advances in Pharmacy and Biotechnology

Journal homepage: http://ijapbjournal.com/

Research Article

Development and validation of LC-MS/MS method for determination of related substances in malathion lotion

Murali Krishna Prasad Vallahbhaneni*, Rajesh Kumar Chawla, Yesu Babu Nalamolu And Rapolu Kishore

Suven Pharmaceuticals Limited, SDE Serene Chambers, Banjara Hills, Hyderabad, Telangana, India 500 034

ARTICLE INFO

Article history:
Received 15 May 2024
Received in revised form 06 June 2024
Accepted 13 June 2024
doi.org/10.38111/ijapb.20241002001

Keywords:
Malathion lotion, LC-MS/MS, Related Substances, Method Validation

ABSTRACT

Malathion is an insecticide of relatively low human toxicity. Malathion in low doses (0.5% w/v) is used as a treatment for head lice, body lice and scabies. Chemically, Malathion is (±)-[(dimethoxyphosphinothioyl)-thio] butanedioic acid diethyl ester. Malathion has a molecular formula of C10H19O6PS2 and molecular weight is 330.36. The reported HPLC method for the estimation of malathion and its impurities in Malathion API was not suitable for Malathion lotion since, the method was unable to show separation between the related substances in Malathion lotion. None of the prior references discloses method for the estimation of related substances in Malathion lotion. Hence, the objective of the present work was to develop and validate a new LC-MS method for determination of related substances in Malathion lotion and validate the method according to ICH guidelines. The method validated in this study was suitable for its intended purpose, which is the quantification of related substances (B, C, D, E, F, G, I, J, L, DEM) in Malathion lotion using Liquid Chromatography with Tandem Mass Spectrometric detection. The method was validated in the range of LOQ-125% for all related substances in Malathion lotion. The method was accurate, linear and precise over the range and no interference was observed in the specificity solutions. The method was robust with respect to the changes in the flow rate of mobile phase. The method was also rugged for an alternative column and different analyst. The stock solutions were stable up to 6 days at room temperature and 2-8°C condition. The proposed method can be successfully applied for the quantification of all related substances in Malathion lotion in routine analysis.

1. Introduction

Malathion is a pediculicide of relatively low human toxicity. Malathion in low doses (0.5% w/v) is used as a treatment for head lice, body lice and scabies [1]. Malathion is an organophosphorus [2-5]. Compound which acts as a pediculicide by inhibiting cholinesterase activity in vivo. Chemically, Malathion is (±)-[(dimethoxyphosphinothioyl)-thio] butanedioic acid diethyl ester. Malathion has a molecular formula of C10H19O6PS2 and molecular weight is 330.36 g/mol. Fig. 1 is the Chemical Structure of Malathion. Malathion is commercially available in the form of lotion containing 0.005 g of Malathion per mL in a vehicle of isopropyl alcohol (78%), terpineol (1.8%), dipentene (1.7%), and pine needle oil (0.04%), [6-9]. Malathion is a clear colorless or slightly yellowish liquid with a characteristic odor.

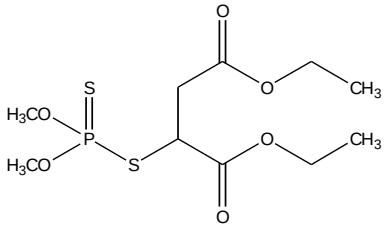

Figure 1: Structure of Malathion

United States Pharmacopoeia disclosed a Liquid Chromatographic method for the assay of Malathion drug substance and estimation of related substance Isomalathion in Malathion drug substance. United States Pharmacopoeia disclosed a Gas Chromatographic method for the assay of Malathion in Malathion lotion drug product. European Pharmacopoeia [10] disclosed a Liquid Chromatographic method for the assay of Malathion

drug substance and estimation of related substance Isomalathion (Impurity-A) and Malaxon (Impurity-B) in Malathion drug substance. British Pharmacopoeia disclosed a Liquid Chromatographic method for the assay of Malathion in Malathion Lotion and estimation of related substance Isomalathion (Impurity A) and Malaxon (Impurity-B) in Malathion lotion drug substance. Visweswariah K et al. [11] reported that a colorimetric method for estimating the malathion residues in rice and wheat samples, involving the use of palladium chloride to form the stable metallic complex, was compared with the standard method of Norris et al. [Journal of Agricultural and Food Chemistry (1954) 2, 570]. Veera Reddy Arava et al. [12] developed a stability indicating RP-HPLC method for the estimation of Malathion drug substance and its impurities. Chuanhong Tu et al. reported [13] that disclose a Gas chromatographic method with Nitrogen-Phosphorus detector for the analysis of organo phosphorus pesticides including Malathion. Abdallah Ouakhssase et.al. Reported [14] that proposed method is ease of use and requires a smaller solvent consumption that reduces the time and cost of the analysis. Malathion monograph is official in European Pharmacopoeia and United States Pharmacopoeia [15] and British Pharmacopoeia [16]. Malathion lotion (0.5%) monograph is official in United States Pharmacopoeia and British Pharmacopoeia.

The reported HPLC method [17] in prior art for the estimation of Malathion and its impurities in Malathion drug substance was not suitable for Malathion lotion. Since, the method was unable to show separation between the related substances in Malathion lotion due to interference of excipients. None of the prior art references discloses method for the estimation of related substances (Impurities-B, C, D, E, F, G, I, J, L & DEM) in Malathion lotion. Hence, the objective of the present work was to develop and validate a new LC-MS/MS method which is more specific for determination of related substances in Malathion lotion and validate the method according to International Council for Harmonization (ICH) guidelines [18-20]. This study propose selection of the method depends on the nature of molecules like ionic, ionizable or neutral, molecular weight and solubility. A method developed and validated in this study is suitable for the Quantitation of related substances (Impurities-B, C, D, E, F, G, I, J, L & DEM) in Malathion lotion using Liquid Chromatography with Tandem Mass Spectrometric detection (LC-MS/MS).

2. Materials and Methods

Malathion, Malathion impurity-B, Malathion impurity-C, Malathion impurity-D, Malathion impurity-E, Malathion impurity-F, Malathion impurity-G, Malathion impurity-I, Malathion impurity-J, Malathion impurity-L, Malathion mono isopropyl ester and Malathion diisopropyl ester were procured from Suven Life Sciences Limited, India. Diethyl maleate and Formic acid were procured from Fluka, India. Methanol (Gradient grade) and Isopropyl alcohol (HPLC grade) were procured from Merck Millipore, India.

The following equipment were used during the study:

Balance - Mettler Toledo AG285

Vortexer - Cole Parmer, Maxi mix-II

Ultra sonicator - Cole Parmer

Micropipettes - Tarson

Water purification System for Ultrapure water type 1 - Milli-Q Millipore system

The method was developed and validated on Shimadzu SIL HTC automated HPLC system equipped with degasser, quaternary pump with gradient mixing, autosampler with temperature control, column compartment with thermostat connected to SCIEX API 3000 - liquid chromatography tandem mass spectrometer (LC-MS/MS). The chromatographic parameters were optimized and optimized chromatographic conditions are given below:

Solution preparation:

Preparation of Reagents:

Mobile phase-A (0.1% Formic Acid):

To 1000 mL reagents bottle add 1000 mL ultra-pure water type-1 and 1000 µL of formic acid and sonicate for 3 minutes.

Mobile phase-B (100% Methanol v/v):

To 1000 mL reagent bottle add 1000 mL of methanol solvent.

Diluent (Isopropyl alcohol, 100% v/v):

To 500 mL reagent bottle add 500 mL of isopropyl alcohol. Diluent shall be used in 30 days.

Standard solution:

Weigh accurately about 2 mg of each impurity-B, C, E, F, G, J & DEM and malathion, 3 mg of impurity-D, 4 mg of impurity-L and 5 mg of impurity-I into a 10 mL volumetric flask containing 5 mL of Isopropyl alcohol, dissolve and makeup the volume with Isopropyl alcohol (solution-A).

Transfer 0.5 mL of above solution-A into 10 mL volumetric flask and makeup the volume with Isopropyl alcohol.

Test solution:

Malathion Lotion neat sample.

Preparation of impurities stock solution (solution-B):

Weigh accurately about 2 mg of each impurity-B, C, E, F, G, J & DEM and Malathion 3 mg of impurity-D, 4 mg of impurity-L and 5 mg of impurity-I into a 10 mL volumetric flask containing 5 mL of Isopropyl alcohol dissolve and makeup the volume with Isopropyl alcohol (solution-B).

Preparation of Malathion solution:

Weight accurately about 50 mg of Malathion in 50 mL volumetric flask containing 20 mL Isopropyl alcohol and make up to the volume with isopropyl alcohol.

Details are shown in Tables 1 to 5 and Fig. 2 to 11.

Table-1: Instrument conditions

Instrumentation	LC-MS/MS (Liquid Chromatography coupled with tandem mass spectrometric detection)
HPLC System	Shimadzu SIL HTC
Mass spectrometer	API 3000-LC-MS/MS (MDS Sciex)
Column	YMC Pack ODS AQ, Length 250 x 4.6 mm, particle size 5 µm
Mobile Phase	Mobile phase-A: 0.1 % formic acid in water Mobile phase-B: Methanol (100% v/v)
Flow rate	1.0 mL/minute
Pumping mode	Binary Flow
Volume of Injection	2 µL
Run time	15 minutes
Sample Solution	Injected impurity mix standard solution

Table-2: Pump Gradient Program

Time (minutes)	Flow rate (mL/minutes)	% Mobile Phase–A	% Mobile Phase–B
00.01	1.0	45	55
2.00	1.0	45	55
8.00	1.0	5	95
10.00	1.0	5	95
12.00	1.0	45	55
15.00	1.0	45	55

Table-3: Mass Detection

Parameter	Positive Mode	Negative Mode
Curtain gas (CUR)	8 psi	8 psi
Collision gas (CAD)	5.0 psi	5.0 psi
Ion Spray Voltage (IS)	5500 V	-3500 V
Temperature (TEM)	250°C	250°C
Nebulizer gas (NEB)	12 psi	12 psi
Resolution	Unit	Unit

Table-4: Impurities and their different types of the potential

Impurity	Declustering Potential	Entrance Potential	Focusing Potential	Collision Energy	Collision cell exit potential	MRM transitions
B	25	9	250	45	2	173.0 to 125.0
C	40	9	250	45	2	156.9 to 109.0
D	40	7	190	30	10	331.0 to 173.1
E	40	11	250	45	2	315.1 to 127.1
F	22	5	210	22	8	303.0 to 271.2
G	50	5	190	50	10	317.0 to 113.2
I*	-5	-10	-90	-35	-12	300.7 to 156.7
J	20	11	180	40	11	345.1 to 127.1
L	60	10	200	20	12	411.1 to 365.1
DEM	14	9	250	25	5	173.0 to 127.1

* Negative ionization mode

Table-5: Retention Time

Impurity	Retention Time (minutes)
B	5.40
C	4.30
D	8.50
E	7.80
F	8.80
G	9.50
I	8.60
J	10.50
L	10.60
DEM	7.10

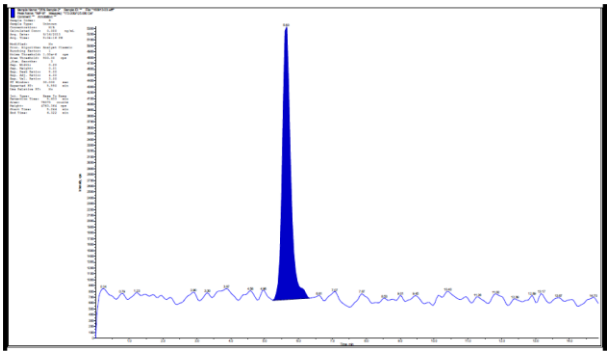

Fig. 2: Representative Chromatogram of Impurity-B with Retention time 5.4 minutes

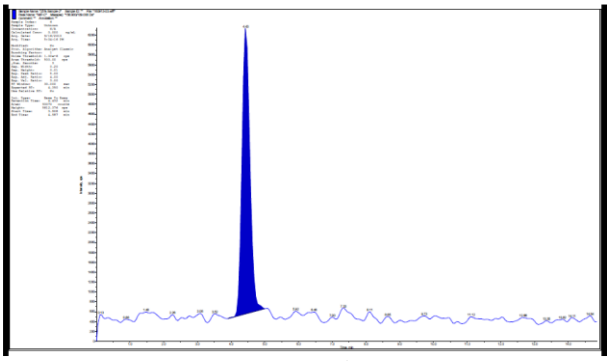

Fig. 3: Representative Chromatogram of Impurity-C with Retention time 4.3 minutes

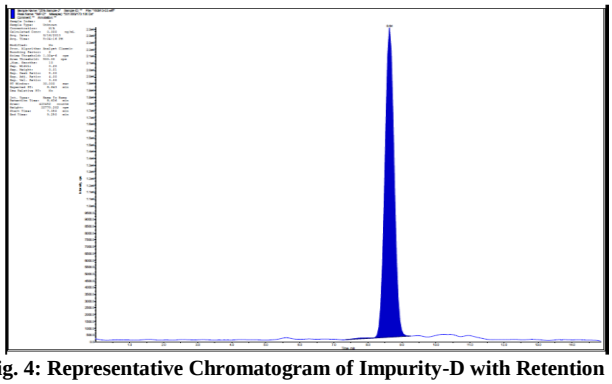

Fig. 4: Representative Chromatogram of Impurity-D with Retention time 8.5 minutes

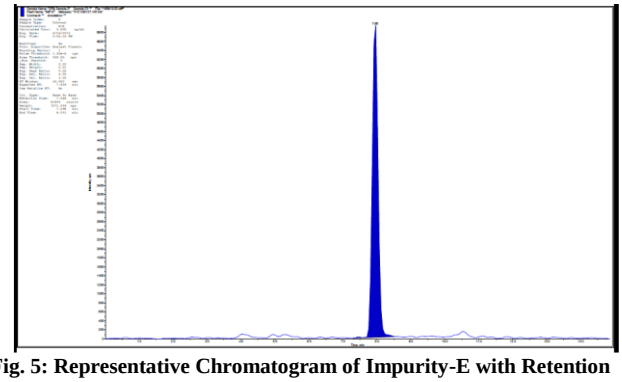

Fig. 5: Representative Chromatogram of Impurity-E with Retention time 7.8 minutes

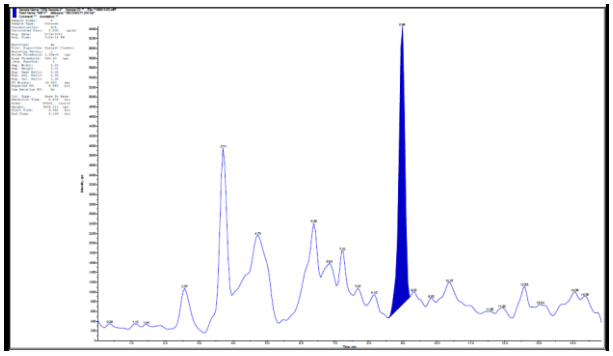

Fig. 6: Representative Chromatogram of Impurity-F with Retention time 8.8 minutes

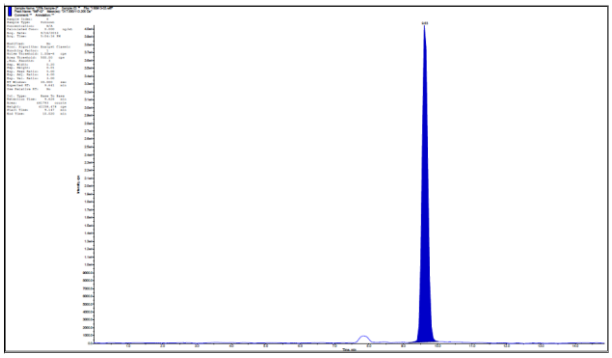

Fig. 7: Representative Chromatogram of Impurity-G with Retention time 9.5 minutes

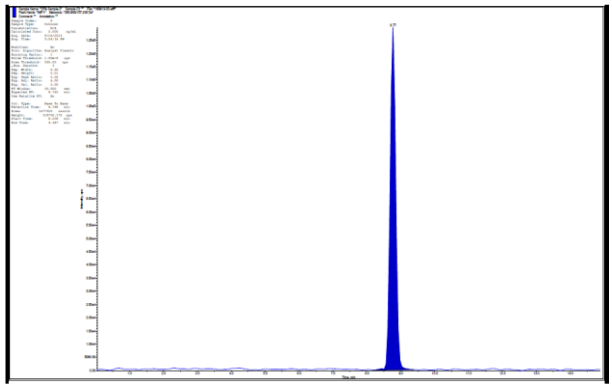

Fig. 8: Representative Chromatogram of Impurity-I with Retention time 8.6 minutes

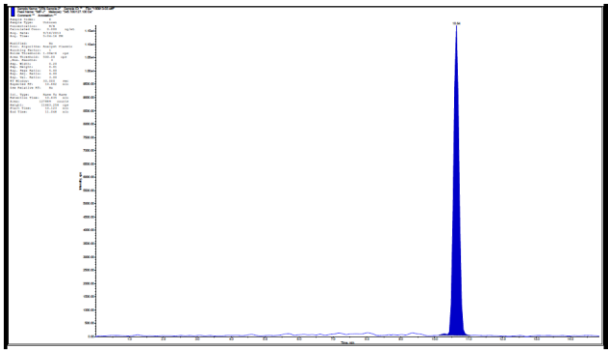

Fig. 9: Representative Chromatogram of Impurity-J with Retention time 10.5 minutes

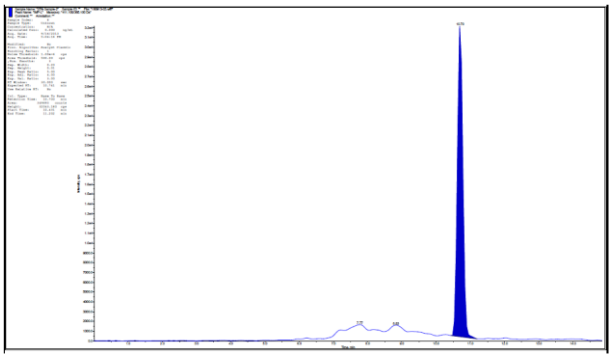

Fig. 10: Representative Chromatogram of Impurity-L with Retention time 10.6 minutes

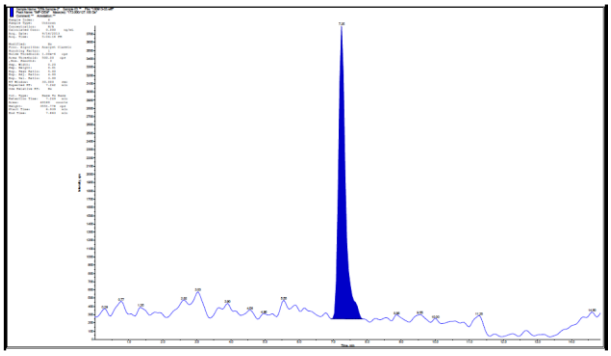

Fig. 11: Representative Chromatogram of Impurity-DEM with Retention time 7.1 minutes

Method Validation:

The developed method was validated as per International Conference on Harmonization (ICH) Q2 (R1) guidelines.

3. Results and Discussion

Specificity:

Objective of specificity is the ability to assess the analyte / impurity unequivocally in the presence of other components, which may be expected to be present, results shown in Table 6.

Acceptance criteria: All the peaks should be identified and integrated in the spiked samples.

Table-6: Specificity Results

Impurity	Retention Time (minutes)	Specification Limit (%)
B	5.43	0.2
C	4.30	0.2
D	8.49	0.3
E	7.77	0.2
F	8.84	0.2
G	9.52	0.2
I	8.63	0.5

J	10.58	0.2
L	10.60	0.4
DEM	7.07	0.2
Malathion mono isopropyl ester	10.50	0.2
Malathion diisopropyl ester	11.00	0.2
Malathion	10.10	NA

Results: All the peaks were identified and integrated in the spiked samples.

Limit of Detection (LOD):

Objective of LOD test is to detect the lowest amount of analyte in a sample but not to quantify as an exact value, results shown in Table 7.

Acceptance criteria: S/N ratio of minimum 3 is required for each injection. The peaks should be detectable.

Table-7: Limit of detection results

Impurity	Concentration (ppm)	S/N Ratio
B	342.09	6.50
C	138.49	6.50
D	101.41	10.70
E	67.13	13.00
F	349.39	6.70
G	70.40	9.50
I	169.68	11.60
J	69.76	6.60
L	137.52	8.30
DEM	355.46	9.20
Malathion	210.75	4.80

Results: The S/N ratio calculated was more than 3 for each injection and the peaks were well detected.

Limit of Quantification (LOQ):

Objective of LOQ test is to quantitation the lowest amount of analyte in a sample with suitable precision and accuracy. In order to determine the limit of Quantitation, a LOQ standard solution containing impurities mixed solution was analyzed in six replicates as shown in Table 8 and 9.

Acceptance criteria: S/N ratio of minimum 10 must be required for each injection. The peaks should be detectable. The RSD of the peak areas should be ≤ 25% for precision at LOQ.

Table-8: Limit of Quantitation results

Impurity	Concentration (ppm)	S/N Ratio
B	1026.28	19.80
C	415.48	21.70
D	304.24	18.90
E	201.38	21.30
F	1048.16	10.80
G	211.21	19.40
I	509.05	17.90
J	209.28	10.00
L	412.57	16.60
DEM	1066.38	18.10
Malathion	632.26	15.60

Table-9: Precision of Limit of Quantitation

Impurity	Average Peak Area	%RSD of Peak Area
B	31725	4.16
C	23136	8.50
D	41694	3.51
E	8196	9.83
F	39085	7.95
G	41863	4.11
I	97454	3.86
J	24179	7.62
L	41553	5.02
DEM	17286	10.23
Malathion	6699	14.40

Results: The S/N ratio calculated was more than 10 for each injection.

The %RSD of the peak areas were less than 25% for precision of LOQ.

Linearity and Range:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the given sample.

For the impurities profile in Malathion lotion, a minimum LOQ to 125% of the specified limits was considered as the range. The linear relationship was evaluated across the range of analytical procedure containing six non-zero standards analyzed for Impurities. The linearity was constructed based on the concentrations obtained by two independent preparations. Results shown in Table 10 and Fig. 12 to 21.

Acceptance criteria: Visual examination of the plot should show good linearity over the range, the correlation coefficient should be ≥ 0.99.

Table-10: Linearity Data of Malathion Related Substances

S. No.	Impurity	Average Area at						Correlation coefficient
		LOQ	25%	50%	75%	100%	125%	
1	B	39071	105141	217916	328192	445830	537444	1.00
2	C	33293	162785	337520	489237	645747	779322	1.00
3	D	56532	558373	1031773	1539413	1953103	2368035	1.00
4	E	12191	99087	172379	269461	348193	427926	1.00
5	F	57910	108791	196343	258744	349200	429226	1.00
6	G	58410	498493	962974	1492883	1841571	2410940	1.00
7	I	92729	898304	1592667	2185991	2744599	3003010	0.99
8	J	27237	713157	1379919	2000772	2533170	3033237	1.00
9	L	51652	480289	885912	1400226	1556770	2088057	0.99
10	DEM	25861	55277	110971	164557	219333	276516	1.00

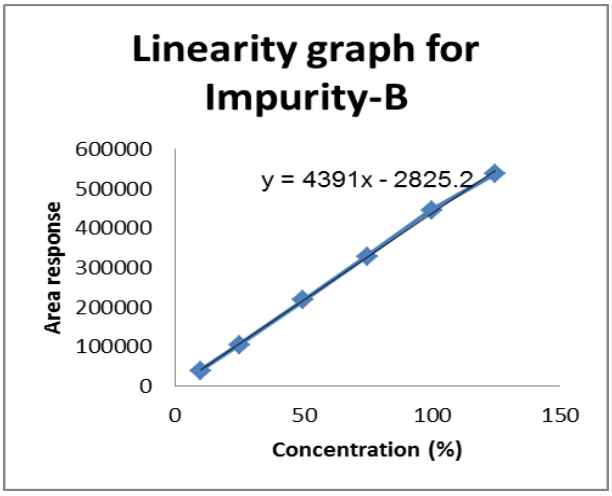

Fig. 12: Malathion impurity-B Linearity graph

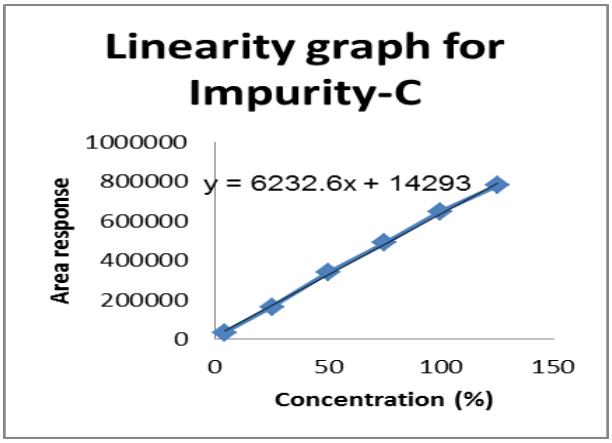

Fig. 13: Malathion impurity-C Linearity graph

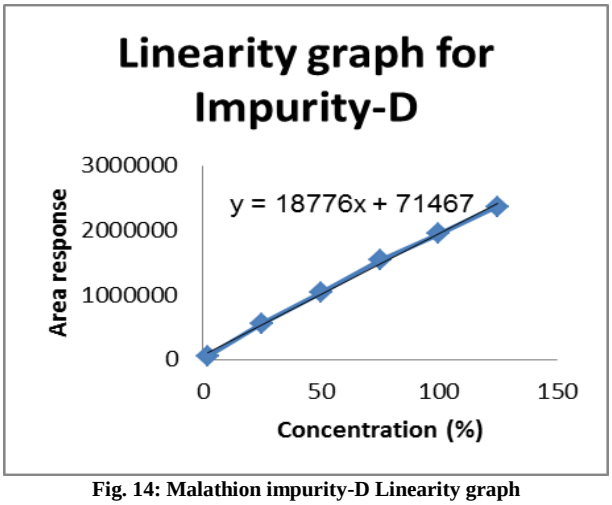

Fig. 14: Malathion impurity-D Linearity graph

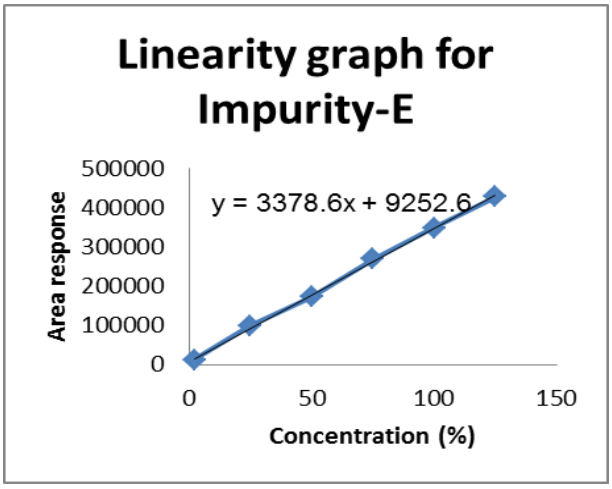

Fig. 15: Malathion impurity-E Linearity graph

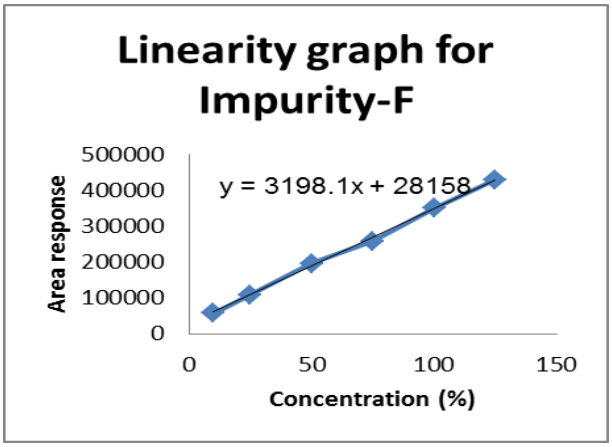

Fig. 16: Malathion impurity-F Linearity graph

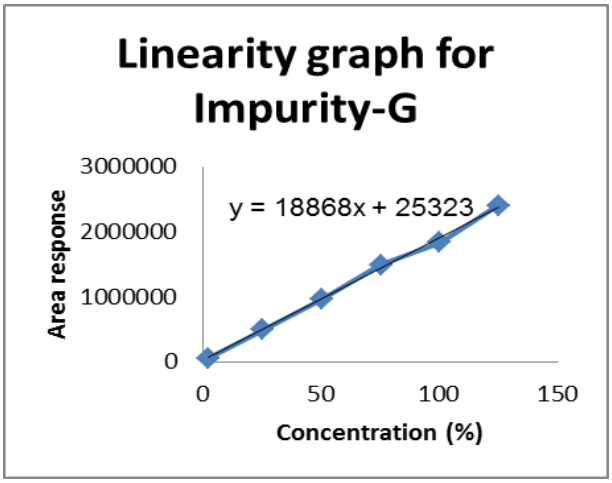

Fig. 17: Malathion impurity-G Linearity graph

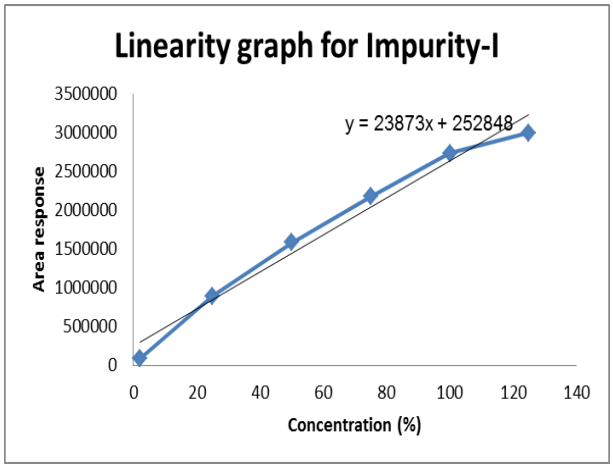

Fig. 18: Malathion impurity-I Linearity graph

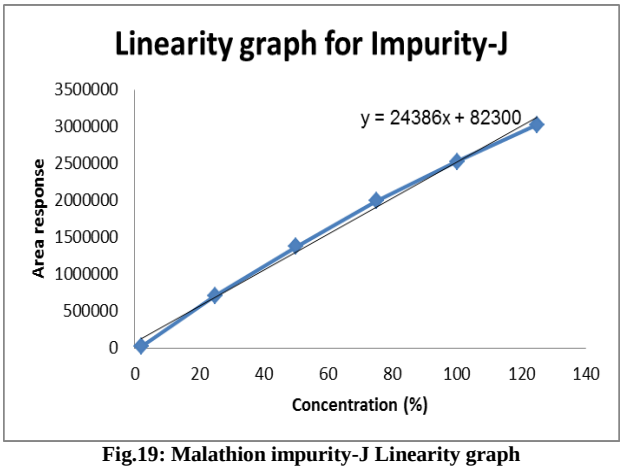

Fig.19: Malathion impurity-J Linearity graph

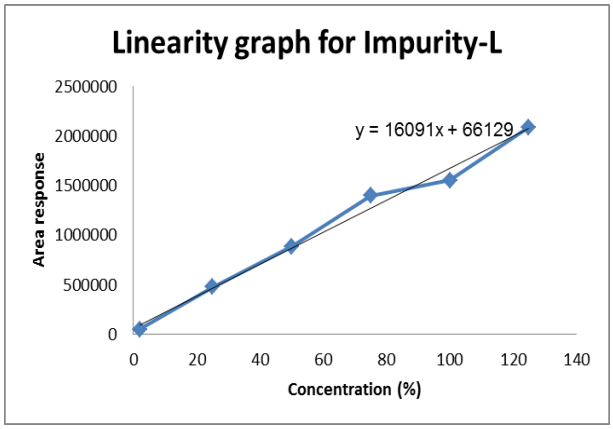

Fig. 20: Malathion impurity-L Linearity graph

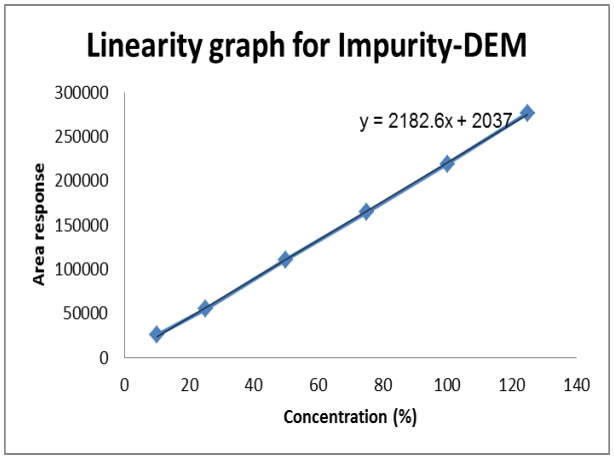

Fig. 21: Malathion impurity-DEM Linearity graph

Results: Plots showed good linearity over the range and the correlation coefficient was ≥ 0.99.

Accuracy (Recovery):

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found. Results are shown in Tables 11 to 21.

The accuracy of the method for impurities will be demonstrated by spiking four different levels of impurities (LOQ, 50%, 100% and 125% of the specification level) to Malathion lotion.

Results: The accuracy of the each concentration level was calculated from the average peak area of thee samples.

The Accuracy was calculated by using the below formula;

$$R = \frac{\text{Corrected area of Impurity}}{\text{Area of the Impurity}} \times 100$$

Where, R = Sample recovery/Accuracy [%],

Corrected area of Impurity = Average area in Spiked Sample-Average area in sample Area of the Impurity = Average area in Standard

Acceptance criteria: The accuracy should be ±20 % for 50%, 100%, 125% solutions and ±30% for LOQ solution.

Table-11: Accuracy (Recovery) results-Impurity-B

Conc (%)	Impurity-B (Peak Area)			Average Peak Area	%RSD
	Solution-1	Solution-2	Solution-3		
LOQ	41391	40356	38582	40110	3.54
50	241527	230958	222330	231605	4.15
100	477840	437888	482637	466122	5.27
125	582362	554706	551230	562766	3.03
LOQ Spiked	41915	44887	44804	43869	3.86
50 Spiked	233350	226478	230252	230027	1.50
100 Spiked	449644	441180	429496	440107	2.30
125 Spiked	543985	531792	520856	532211	2.17

Table-12: Accuracy (Recovery) results-Impurity-C

Conc (%)	Impurity-C (Peak Area)			Average Peak Area	%RSD
	Solution-1	Solution-2	Solution-3		
LOQ	31940	29186	34505	31877	8.34
50	370775	361750	366347	366291	1.23
100	732947	702356	736223	723842	2.58
125	889255	882033	865563	878950	1.38
LOQ Spiked	39788	34964	36057	36936	6.85
50 Spiked	364724	378470	372019	371738	1.85
100 Spiked	682717	671982	663102	672600	1.46
125 Spiked	773392	815519	827223	805378	3.52

Table-13: Accuracy (Recovery) results-Impurity-D

Conc (%)	Impurity-D (Peak Area)			Average Peak Area	%RSD
	Solution-1	Solution-2	Solution-3		
LOQ	68825	63209	60834	64289	6.38
50	1244360	1252513	1216563	1237812	1.52
100	2350291	2344320	2391592	2362068	1.09
125	2860628	2836821	2711008	2802819	2.87
LOQ Spiked	129340	130906	123417	127888	3.09
50 Spiked	1361589	1294252	1273407	1309749	3.52
100 Spiked	2376989	2265404	2275365	2305919	2.68
125 Spiked	2727104	2579942	2557447	2621498	3.52

Table-14: Accuracy (Recovery) results-Impurity-E

Conc (%)	Impurity-E (Peak Area)			Average Peak Area	%RSD
	Solution-1	Solution-2	Solution-3		
LOQ	14083	15089	14657	14850	5.92
50	210733	237928	209088	219250	7.39
100	386925	459058	433528	426504	8.58
125	511860	530482	481069	507804	4.91
LOQ Spiked	23940	26158	25597	25232	4.57
50 Spiked	245540	224446	220416	230134	5.86
100 Spiked	462029	422475	407747	430750	6.52
125 Spiked	502409	486180	510855	499815	2.51

Table-15: Accuracy (Recovery) results-Impurity-F

Conc (%)	Impurity-F (Peak Area)			Average Peak Area	%RSD
	Solution-1	Solution-2	Solution-3		
LOQ	43113	44657	42876	43549	2.22
50	186854	180095	184230	183726	1.85
100	367953	350172	365398	361174	2.66
125	463936	440802	437259	447332	3.24
LOQ Spiked	49416	44484	49481	47794	6.00
50 Spiked	187099	186081	177781	183654	2.78
100 Spiked	384783	350677	352641	362700	5.28
125 Spiked	446000	396231	400387	414206	6.67

Table-16 Accuracy (Recovery) results-Impurity-G

Conc (%)	Impurity-G (Peak Area)			Average Peak Area	%RSD
	Solution-1	Solution-2	Solution-3		
LOQ	64959	61621	63855	63478	2.68
50	1237959	1225235	1201700	1221631	1.51
100	2338883	2303220	2438103	2360069	2.96
125	2880453	2788703	2698857	2789338	3.26
LOQ Spiked	444855	445170	440894	443640	0.54
50 Spiked	1517852	1589313	1603061	1570075	2.91
100 Spiked	2837607	2598386	2586716	2674236	5.30
125 Spiked	3054119	2892889	3014096	2987035	2.81

Table-17: Accuracy (Recovery) results-Impurity-I

Conc (%)	Impurity-I (Peak Area)			Average Peak Area	%RSD
	Solution-1	Solution-2	Solution-3		
LOQ	94382	99552	103979	99304	4.84
50	1513963	1546577	1568660	1543067	1.78
100	2551002	2672034	2871668	2698235	6.00
125	3175860	2997735	2933916	3035837	4.13
LOQ Spiked	98504	104084	111808	104799	6.37
50 Spiked	1579112	1548834	1473534	1533827	3.54
100 Spiked	2642474	2408275	2483961	2511570	4.76
125 Spiked	2918793	2759812	2637573	2772059	5.09

Table-18: Accuracy (Recovery) results-Impurity-J

Conc (%)	Impurity-J (Peak Area)			Average Peak Area	%RSD
	Solution-1	Solution-2	Solution-3		
LOQ	33237	35045	31378	33220	5.52
50	1623406	1623274	1571431	1606037	1.87
100	3037817	3034739	2952840	3008465	1.60
125	3611132	3490203	3325264	3475533	4.13
LOQ Spiked	512480	540048	510585	521038	3.16
50 Spiked	1930269	1994103	1893892	1939421	2.62
100 Spiked	3179636	3186407	3145223	3170422	0.70
125 Spiked	3572960	3703620	3441919	3572833	3.66

Table-19: Accuracy (Recovery) results-Impurity-L

Conc (%)	Impurity-L (Peak Area)			Average Peak Area	%RSD
	Solution-1	Solution-2	Solution-3		
LOQ	77947	73754	69395	73699	5.80
50	1272625	1402350	1163346	1279440	9.35
100	2335764	2528919	2268944	2377876	5.68
125	2868139	2851529	2762798	2827489	2.00
LOQ Spiked	380087	418628	425557	408091	6.00
50 Spiked	1557172	1525812	1602290	1561758	2.46
100 Spiked	2578157	2536556	2611026	2575246	1.45
125 Spiked	2694602	2765876	2869420	2776633	3.17

Table-20: Accuracy (Recovery) results-Impurity-DEM

Conc. (%)	Impurity-DEM (Peak Area)			Average Peak Area	%RSD
	Solution-1	Solution-2	Solution-3		
LOQ	31175	29233	23937	28115	13.32
50	112160	115259	118242	115220	2.64
100	246139	259101	235119	246786	4.86
125	293675	312172	264751	290199	8.24
LOQ Spiked	22679	28679	30143	27167	14.56
50 Spiked	120241	111646	114367	115418	3.81
100 Spiked	223433	234045	244574	234014	4.52
125 Spiked	282169	281281	284945	282798	0.68

Table-21: %Recovery of impurities (B,C,D,E,F,G,I,J,L and DEM)

Impurity	%Recovery			
	LOQ	50%	100%	125%
B	109.37	99.32	94.42	94.57
C	115.87	101.49	92.92	91.63
D	107.48	101.06	95.13	91.43
E	102.94	100.43	98.66	96.47
F	109.75	99.96	100.42	92.59
G	122.92	98.59	97.82	93.98
I	105.53	99.40	93.08	91.31
J	119.79	90.79	89.39	88.95
L	124.04	97.31	94.98	87.00
DEM	96.63	100.17	94.82	97.45

Results: All the accuracy results for the four levels were in compliance to acceptance criteria.

System Precision:

System precision was performed as routinely at the beginning of each day by injecting six replicate of Malathion and impurities mixed solution at a concentration of 100% standard level. The purpose of the system precision was to check the chromatographic conditions by evaluating the retention time, peak shape and area response of the analyte. Results are shown in Table 22.

Method Precision:

Method precision was performed by injecting six preparations of impurities mixed solution spiked to Malathion lotion at a concentration of 100% standard level. The purpose of method precision was to check the chromatographic conditions by evaluating the retention time, peak shape and area response of the analyte. Results are shown in Table 23.

Ruggedness:

Intermediate precision:

To evaluate the intermediate precision, repeated the method repeatability study on a different day, using a different lot of column and different analyst. Results are shown in Table 24.

Acceptance criteria:

•%RSD for the areas of each impurity should not be more than 15.

•Overall %RSD for the areas for normal and modified conditions should not be more than 20.

Results: The results were found to be within the acceptance criteria.

Robustness:

The evaluation of robustness was done by injecting the system precision solution at the original method condition and the variable method conditions. Results are shown in Table 25.

Table-22: System precision results

Day	Impurity	Mean Peak Area	%RSD of Peak Area	Mean RT	%RSD of RT
Day-1	B	278538	5.62	5.44	0.52
	C	354030	6.38	4.30	0.09
	D	1376783	7.75	8.51	0.47
	E	216113	8.59	7.79	0.56
	F	209084	7.54	8.86	0.38
	G	1368676	6.99	9.53	0.17
	I	2182671	2.48	8.65	0.41
	J	2006184	8.58	10.53	0.49
	L	1282209	14.74	10.60	0.00
	DEM	140643	8.09	7.09	0.57
	Malathion	71514	7.33	10.10	0.00
Day-2	B	286640	3.98	5.48	0.00
	C	362315	1.12	4.32	0.32
	D	1320560	2.84	8.52	0.28
	E	215829	5.86	7.81	0.10
	F	237404	6.23	8.87	0.10
	G	1230216	5.44	9.54	0.07
	I	1842649	2.57	8.66	0.06
	J	2018305	4.46	10.55	0.52
	L	1217997	7.55	10.60	0.00
	DEM	142952	6.60	7.11	0.14
	Malathion	73010	4.22	10.10	0.00
Day-3	B	288179	1.56	5.49	0.12
	C	377607	2.93	4.34	0.09
	D	1280175	3.83	8.52	0.05
	E	193459	4.55	7.82	0.05
	F	228385	8.20	8.88	0.00
	G	1160345	6.30	9.54	0.00
	I	1563841	2.01	8.66	0.00
	J	1963221	2.36	10.60	0.00
	L	1249901	3.08	10.60	0.00
	DEM	131325	3.71	7.12	0.06
	Malathion	72848	3.04	10.10	0.00
Day-4	B	384632	3.66	5.55	2.15
	C	586743	4.32	4.39	2.44
	D	1558425	5.40	8.55	0.16
	E	250684	5.01	7.85	0.87
	F	264103	8.98	8.90	0.53
	G	1427989	4.37	9.56	0.36
	I	1681828	2.27	8.69	0.57
	J	2346944	3.44	10.60	0.00
	L	1464041	4.40	10.62	0.38
	DEM	169524	4.63	7.16	1.21
	Malathion	70356	6.54	10.10	0.00
Day-5	B	409406	3.23	5.52	0.07
	C	628546	2.36	4.36	0.00
	D	2011200	4.19	8.54	0.05
	E	367515	4.62	7.84	0.00
	F	330901	3.57	8.89	0.00
	G	2030375	4.78	9.55	0.05
	I	1118338	1.98	8.68	0.00
	J	2584072	2.62	10.60	0.00
	L	2135344	4.54	10.60	0.00
	DEM	201996	2.92	7.14	0.07
	Malathion	60067	3.65	10.10	0.00
Day-6	B	414476	2.79	5.52	0.32
	C	600258	3.64	4.36	0.28
	D	2018684	3.12	8.54	0.14
	E	380470	2.45	7.83	0.26
	F	333461	3.48	8.89	0.14

Day	Impurity	Mean Peak Area	%RSD of Peak Area	Mean RT	%RSD of RT
	G	2177498	4.15	9.22	0.13
	I	2040510	2.20	8.67	0.18
	J	2736286	1.42	10.53	0.49
	L	2020439	9.64	10.60	0.00
	DEM	214540	2.03	7.14	0.29
	Malathion	66321	5.35	10.10	0.00
Day-7	B	282322	2.38	5.51	0.00
	C	367713	3.48	4.35	0.00
	D	1558931	3.16	8.55	0.07
	E	269265	5.44	7.84	0.08
	F	246010	2.97	8.90	0.09
	G	1552186	4.79	9.56	0.07
	I	1621146	2.18	8.69	0.07
	J	2095233	4.74	10.60	0.00
	L	1668127	6.77	10.63	0.49
	DEM	144741	3.97	7.14	0.06
	Malathion	74698	5.71	10.10	0.00

Table-23: Method precision results

Impurity	Mean Peak Area	%RSD of Peak Area	Mean RT	%RSD of RT
B	394247	2.96	5.42	0.10
C	605384	2.74	4.30	0.09
D	1922767	4.20	8.49	0.06
E	333848	4.03	7.77	0.05
F	335006	5.66	8.85	0.06
G	2312449	4.84	9.52	0.04
I	1951275	2.58	8.63	0.00
J	2624456	4.11	10.52	0.39
L	1702738	3.13	10.60	0.00
DEM	206453	7.65	7.07	0.07

Table-24: Intermediate precision Result

Day	Impurity	Mean Peak Area	%RSD of Peak Area	Overall %RSD for peak area	Mean RT	%RSD of RT
Day-1	B	324044	4.59	9.73	5.52	0.10
	C	414068.3	6.55	8.39	4.35	0.09
	D	17017314	4.35	6.42	8.55	0.00
	E	277792.7	3.67	2.20	7.84	0.05
	F	270381.7	5.79	6.67	8.90	0.06
	G	1672490	5.39	5.28	9.57	0.06
	I	1583467	6.44	1.66	8.69	0.05
	J	2275258	5.04	5.83	10.60	0.00
	L	1770242	3.62	4.20	10.65	0.51
	DEM	159590.2	4.01	6.90	7.14	0.07
	Malathion	86792.67	5.92	9.73	10.10	0.00
Day-2	B	462196	2.69	7.70	5.52	0.07
	C	700890	2.95	10.94	4.36	0.09
	D	2246671	5.67	7.56	8.53	0.06
	E	418551	8.36	6.74	7.83	0.00
	F	395349	5.75	12.01	8.89	0.06
	G	248558	7.98	5.34	9.55	0.04
	I	2023001	7.09	0.61	8.67	0.05
	J	2928676	4.53	4.80	10.60	0.00
	L	2302240	4.86	9.22	10.60	0.00
	DEM	230298	4.20	5.01	7.14	0.06
	Malathion	67971	3.07	7.70	10.10	0.00

Table-25: Robustness Results

Parameter	Impurity	Mean Peak Area	%RSD of Peak Area	Overall %RSD for peak area	Mean RT	%RSD of RT
Flow rate 0.900 mL/minutes	B	494259	4.29	10.08	5.99	0.09
	C	755816	6.09	13.20	4.75	0.00
	D	2527639	4.47	12.79	9.03	0.05
	E	444958	3.88	8.01	8.32	0.00
	F	432245	4.86	9.61	9.39	0.05
	G	2526284	7.21	7.49	10.10	0.00
	I	2091190	4.59	9.17	9.17	0.06
	J	3023334	4.86	5.06	11.10	0.00
	L	2347945	3.46	8.87	11.20	0.00
	DEM	256622	3.42	11.39	7.64	0.05
	Malathion	81447	5.86	13.58	10.60	0.00
Flow rate 1.100 mL/minutes	B	419669	5.10	10.08	5.99	0.09
	C	612132	4.03	13.20	4.75	0.00
	D	2063797	8.13	12.79	9.03	0.05
	E	402482	7.43	8.01	8.32	0.00
	F	464059	8.48	9.61	9.39	0.05
	G	2320733	9.13	7.49	10.10	0.00
	I	1757161	8.27	9.17	9.17	0.06
	J	2842912	10.10	5.06	11.10	0.00
	L	2020831	10.20	8.87	11.20	0.00
	DEM	209610	6.72	11.39	7.64	0.05
	Malathion	63719	9.66	13.58	10.60	0.00

Table-26: Stability Results

Impurity	% Relative Standard Deviation Acceptance criteria not more than 15%					
	Benchtop Stability (24 Hrs)	Refrigerated Stability (24 Hrs)	Benchtop Stability (48 Hrs)	Refrigerated Stability (48 Hrs)	Benchtop Stability (6 days)	Refrigerated Stability (6 days)
B	4.49	3.95	2.52	3.67	3.35	1.91
C	3.15	3.19	2.23	2.21	3.68	3.81
D	4.47	4.68	2.61	3.86	4.45	6.71
E	7.67	4.99	3.18	4.77	2.54	6.46
F	2.57	3.95	1.70	3.59	5.35	7.46
G	6.56	5.23	2.67	3.80	6.33	6.49
I	4.60	1.55	1.42	3.03	4.29	4.46
J	2.37	2.38	1.72	3.46	4.65	4.88
L	7.27	4.21	5.19	7.65	3.31	5.25
DEM	1.80	2.39	3.68	4.79	3.37	4.72

The parameters tested were the influence of variations flow rate at ±0.1 mL of the original flow rate (i.e. 900 µL/minutes and 1100 µL/minutes).

Acceptance criteria:

%RSD calculated for retention time without omitting any data from all six injections should not be more than 5%.

%RSD calculated for area response without omitting any data from all six injections should not be more than 15%.

Results: Robustness results for the variable conditions were in compliance to acceptance criteria.

Stability of Stock Solutions:

The stability of the system suitability solution, over the time was demonstrated at 24 hours, 48 hours and 6 Days in Bench top and refrigerated condition. The test was performed Keep the impurities spiked sample solution (six preparations) at bench top for 24 hours, 48 hours and 6 days. Results are shown in Table 26.

Results: The stock solutions were stable up to 6 days at room temperature and refrigerated condition.

4. Conclusion

•Successfully developed a new method which is not present in prior art and is specific, avoids all interferences due to excipients for the determination of related substances in Malathion lotion drug product and presented good linearity, specificity, accuracy, precision and robustness.
•The method validated in this study is suitable for the Quantitation of related substances (Impurity-B, C, D, E, F, G, I, J, L & DEM) in Malathion lotion using Liquid Chromatography with Tandem Mass Spectrometric detection (LC-MS/MS).
•The method was validated in the range of LOQ-125% for related substances (Impurity-B, C, D, E, F, G, I, J, L & DEM) in Malathion lotion. The method was accurate, linear and precise over the range and no interference was observed in the specify solutions.
•The method was robust with respect to the changes in the flow rate of mobile phase. The method was also rugged for an alternative column and different analyst.
•The stock solutions were stable up to 6 days at room temperature and refrigerated conditions.
•The analytical method is stability indicating and can be successfully applied for the Quantitation of related substances (Impurity-B, C, D, E, F, G, I, J, L & DEM) in Malathion lotion and may be used for other drug products, where present excipients interaction with related substances.

Acknowledgements

The authors express thanks to Suven Pharmaceuticals Limited, Hyderabad for support and providing the research facility to carrying out the work.

Conflict of Interest

The author(s) confirm that this article content has no conflict of interest.

References

1. Carpenter J, Weil C, Palm W, Woodside MD, Smyth HF Jr. Acute toxicity of some agricultural chemicals. J. Agric. Food Chem., 1960; 8(1): 43-47.
2. Aldridge WN, et al. Interactions between organophosphorus compounds and the metabolism of testosterone. Arch. Toxicol., 1979; 42: 95-106.
3. Boutsiouski P, et al. The influence of oxygen tension on cytotoxicity. Arch. Toxicol., 2001; 75(6): 321-328.
4. Bouchard M, et al. Effects of prenatal methylmercury exposure on the developing brain. Toxicol. Sci., 2003; 73: 182-194.
5. Padilla S, et al. Differential sensitivity of male and female rat liver to oxidative stress. J. Toxicol. Environ. Health, Part A, 2004; 67: 1477-1489.
6. Norris MV, Vail WA, Averell PR. Study of natural insecticidal properties in plants. J. Agric. Food Chem., 1954; 2(11): 570-573.
7. Dionísio AP, Pastore GM. The impact of natural antioxidants on the stability of foods. In: Natural Food Additives, Ingredients and Flavourings. 2012..
8. O'Neil MJ. The Merck Index. 14th ed. Whitehouse Station, NJ: Merck & Co.; pp. 985-986.
9. Sweetman SC. Martindale: The Complete Drug Reference. 35th ed. London, UK: Pharmaceutical Press; pp. 1862.
10. European Pharmacopoeia. 8th ed. 2014; pp. 2685.
11. Visweswariah K, Jayaram M. Effects of pesticides on enzyme activities in crops. Agric. Biol. Chem., 1974; 38(10): 2031-2033.
12. Reddy AV, Reddy BM, Rao CK, Gopichand T, Reddy CS. Evaluation of pharmaceutical excipients. Der Pharma Chem., 2013; 5(2): 14-18.
13. Tu C. Analysis of organ phosphorus pesticides with Agilent 6820 Gas chromatograph/Nitrogen Phosphorus detector. In: Environmental and Food Analysis. Agilent Technologies; August 13, 2004.
14. Ouakhssase A, Chahid A, Choubbane H, Aitmazirt A, Addi EA. Investigation of food contaminants. Heliyon, 2019; e01565.
15. United States Pharmacopoeia. 38th ed. 2015; pp. 4193-4195.
16. British Pharmacopoeia. 2016; pp. 182-183, 798-799.
17. International Conference on Harmonization (ICH) Guideline on Stability Testing of New Drug Substances and Products. Q1A (R2), 2003.
18. International Council for Harmonization (ICH) Guideline on Photo Stability Testing of New Drug Substances and Products. Q1B, 1996.
19. International Council for Harmonization (ICH) Guideline on Impurities in New Drug Substances and Products. Q3B (R2), 2006.
20. International Council for Harmonization (ICH). ICH Q2(R1), Validation of Analytical Procedures: Text and Methodology, 2005.