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A Validated Stability Indicating GC-NPD Method for Determination of Related Substances in Malathion Lotion

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A versatile stability indicating analytical method was developed and validated for determination of closely related impurities of malathion *viz.*, malathion impurity J, malathion mono isopropyl ester and malathion di isopropyl ester using gas chromatography with nitrogen phosphorous detector (GC-NPD) in malathion lotion. The chromatographic separation was performed using a column with G1 stationary phase (dimethylpolysiloxane). As part of the method validation, specificity, limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy, precision, robustness and ruggedness were determined. The limit of quantification values for malathion Impurity J, malathion mono isopropyl ester and malathion di isopropyl ester were 0.016, 0.012 and 0.009 %, respectively. Good linearity ($r^2 > 0.99$) was obtained ranging from limit of quantification to 150 %. Recovery was verified for all the three impurities at concentrations ranging from limit of quantification to 150 %. Percent recovery for malathion impurity J, malathion mono isopropyl ester and malathion di isopropyl ester were 104.2 to 113.5 %, 99.4 to 106.4 % and 97.4 to 114.1 % respectively. The admissible robustness indicates that the method remain unaffected by small but deliberate variations. Malathion was found to degrade in acid and base stress conditions. This method has been successfully applied to pharmaceutical lotion formulation and no interference for the excipients was found.

Keywords: Malathion, Malathion lotion, Related impurities, GC-NPD method, Validation.

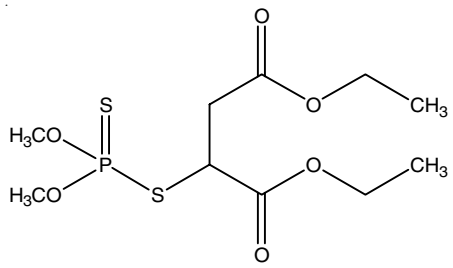
INTRODUCTION

Malathion is an insecticide of relatively low human toxicity. Malathion in low doses (0.5 % preparations) is used as a treatment for head lice, body lice and scabies [1]. Malathion is an organophosphate agent which acts as a pediculocide by inhibiting cholinesterase activity *in vivo*. Chemically, malathion is (±)-[(dimethoxyphosphinothioyl)thio]butanedioic acid diethyl ester. Malathion has a molecular formula of $C_{10}H_{19}O_6PS_2$ and molecular weight of 330.36. 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, dipentene and pine needle oil [2,3]. Malathion is a clear colourless or slightly yellowish liquid with a characteristic odour [4,5]. Malathion is official in European Pharmacopoeia [6], United States Pharmacopoeia [7] and British Pharmacopoeia [8]. Malathion lotion is official in United States Pharmacopoeia [7] and British Pharmacopoeia [8].

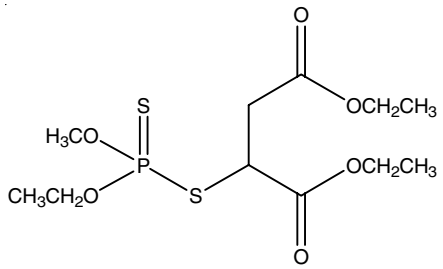
Literature survey revealed a colorimetric method for the estimation of malathion [9], RP-HPLC method with UV detector [10] for the estimation of malathion impurities in malathion lotion. The aim of the present work was to develop and validate a new GC-NPD method for determination of closely related impurities of malathion [Fig. 1(a)] *viz.*, malathion impurity J [Fig. 1(b)], malathion monoisopropyl ester impurity [Fig. 1(c)] and malathion diisopropyl ester impurity [Fig. 1(d)] in malathion lotion, since the existing USP method for the estimation of malathion using gas chromatography [7] was not suitable for estimation of all the plausible impurities in malathion lotion. The developed method was validated in accordance with International Conference on Harmonization (ICH) guidelines [11-13].

EXPERIMENTAL

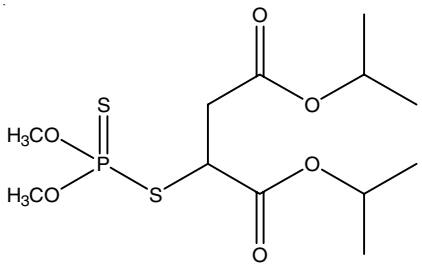
Malathion and its impurities *viz.* impurity-A, impurity-B, impurity-C, impurity-D, impurity-E, impurity-F, impurity-G, impurity-H, impurity-I, impurity-J (O,O-methyl ethyl S-(1,2-dicarboethoxy)ethyl phosphorodithoate), impurity-K, impurity-L, malathion mono isopropyl ester and malathion di isopropyl ester and excipients such as α-terpineol, dipentene, pine needle oil were obtained from Suven Life sciences Ltd., Hyderabad, India. In addition, analytical reagent grade ethyl acetate and isopropyl alcohol was purchased from Merck, (Mumbai, India). All chemicals and solvents were of analytical grade. The chemical names of the impurities of malathion were given in Table-1.



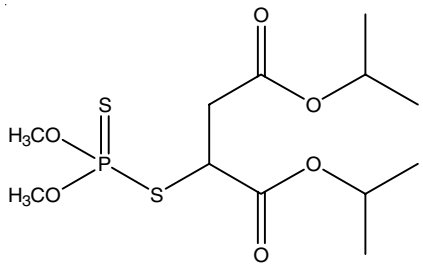
(a) Malathion: (±)-[(dimethoxyphosphinothioyl)thio]butanedioic acid diethyl ester; m.f.: $C_{10}H_{19}O_6PS_2$; m.w.: 330.36



(b) Malathion impurity-J: O,O-methyl ethyl S-(1,2-dicarboethoxy)ethyl phosphorodithioate; m.f.: $C_{11}H_{21}O_6S_2P$; m.w.: 344.58



(c) Malathion mono isopropyl ester impurity: 2-[(dimethoxyphosphinothioyl)thio]butanedioic acid ethyl isopropyl ester; m.f.: $C_{11}H_{21}O_6PS_2$; m.w.: 344.38



(d) Malathion di isopropyl ester impurity: 2-[(dimethoxyphosphinothioyl)thio]butanedioic acid diisopropyl ester; m.f.: $C_{12}H_{23}O_6PS_2$; m.w.: 358.41

Fig. 1. Chemical structures of malathion and its impurities

TABLE-1
CHEMICAL NAMES OF MALATHION IMPURITIES

Impurity Name	Chemical Name
Impurity-A	O,O,S-Trimethyl phosphorodithioate
Impurity-B	O,S,S-Trimethyl phosphorodithioate
Impurity-C	Trimethylphosphorothioate
Impurity-D	Isomalathion
Impurity-E	Malaoxon
Impurity-F	Dimethyl malathion
Impurity-G	Methyl malathion
Impurity-H	Diethyl fumarate
Impurity-I	O,O-dimethyl-S-(1-carboxy-2-carboethoxy)ethyl phosphorodithioate
Impurity-J	O,O-methyl ethyl S-(1,2-dicarboethoxy)ethyl phosphorodithioate
Impurity-K	Diethyl 2-mercaptosuccinate
Impurity-L	Tetraethyl Dithiosuccinate
Diethyl maleate	Diethyl maleate
Malathion mono isopropyl ester	2-[(Dimethoxyphosphinothioyl)thio]butanedioic acid ethyl isopropyl ester
Malathion di isopropyl ester	2-[(Dimethoxyphosphinothioyl)thio]butanedioic acid ethyl di isopropyl ester

Diluent: Ethyl acetate was used as a diluent.

Preparation of standard stock solutions: 5 mg each of malathion, malathion impurity-J, malathion mono isopropyl ester, malathion di isopropyl ester were weighed accurately and dissolved in 10 mL of ethyl acetate in a 25 mL volumetric flask and the volume was made upto 25 mL using ethyl acetate.

Preparation of standard solutions: Further, 0.5 mL of the prepared stock solution was transferred into a 20 mL volumetric flask and diluted the volume upto the mark with ethyl acetate.

Preparation of sample solution: 5 mL of the test solution was transferred into 10 mL volumetric flask and diluted the volume upto the mark with ethyl acetate.

Placebo preparation: Transferred 1.1 mL of α-terpinol, 1 mL of dipentene and 0.025 mL of pineneedle oil in 10 mL volumetric flask and diluted the volume up to the mark with isopropyl alcohol. Transferred 5 mL of above solution in to 10 mL volumetric flask and diluted the volume up to the mark with ethyl acetate.

Instrumentation: The chromatographic analysis was performed by an Agilent gas chromatography system equipped with a nitrogen and phosphorus detector (NPD) and using nitrogen as carrier gas.

Method development

Optimization of chromatographic conditions: Proper selection of method depends on the nature of the drug molecule like ionic, ionizable and neutral and its molecular weight and solubility. The reported HPLC method [10] for determination of impurity profile in malathion lotion was unable to show separation between the three impurities malathion impurity J, malathion mono isopropyl ester and malathion di isopropyl ester. This may be due to the close structural similarities and other properties of these impurities with malathion. Hence, it

was proposed to develop an analytical method for the determination of these impurities by gas chromatography technique. Initially gas chromatographic conditions were selected based on the literature for the analysis of malathion using gas chromatograph with nitrogen phosphorus detector [14].

For the optimization of the chromatographic conditions, different trials were performed using different columns (DB-FFAP, HP-5, ZB-Multi residue-1, EC-5 and AT-1) and at different conditions to achieve the best method using nitrogen phosphorus detector.

Optimized chromatographic conditions: Chromatographic separation was achieved using an AT-1 capillary column (60 m × 0.25 mm ID, 1.0 µm). Hydrogen (3 mL min^{-1}) and dried air (60 mL min^{-1}) were used as auxiliary gases for the detector (NPD). Nitrogen was used as a carrier gas with a constant pressure of 36.4 psi. The injection volume was 0.2 µL. The injector and detector temperatures were maintained at 250 and 340 °C respectively. The oven temperature program was as follows: initial temperature maintained at 120 °C for 0.5 min, raised to 190 °C at a rate of 15 °C min^{-1}, maintained for 5 min, then raised to 230 °C at a rate of 5 °C min^{-1}, maintained for 20 min and raised to final temperature of 280 °C at a rate of 25 °C min^{-1}, where the temperature was maintained for 10 min. The total run time of analysis was 50.2 min. Ethyl acetate was used as the diluent. The chromatograms obtained in these operating conditions for diluent, placebo solution and standard solutions of malathion and its impurities were shown in Fig. 2(a), 2(b) and 2(c) respectively and the retention times for malathion, malathion mono isopropyl ester, malathion impurity J and malathion di isopropyl ester were 34.7, 36.5, 37.3 and 38.1 min, respectively.

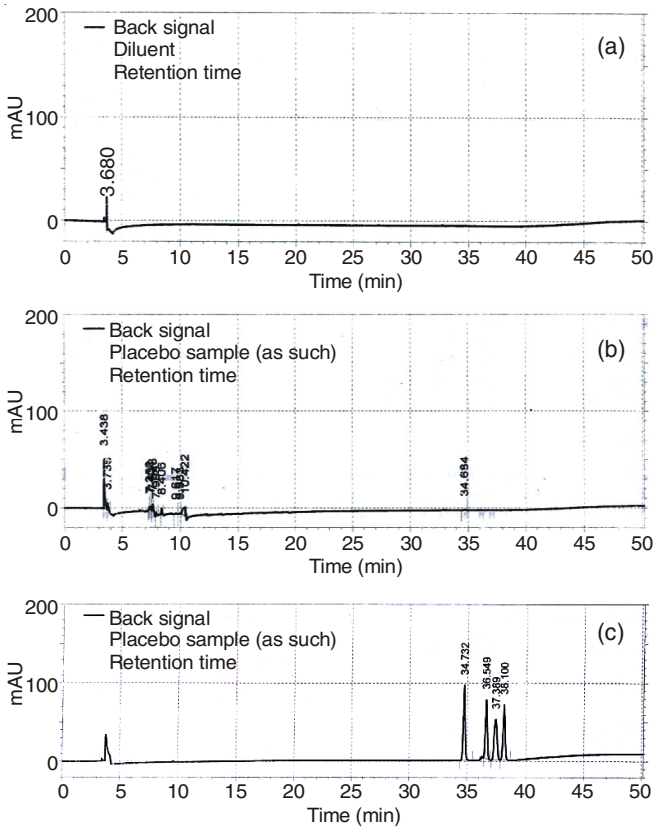


Fig. 2. Standard chromatogram for diluent (a), placebo solution (b) and standard solutions (c)

Method validation: To demonstrate the feasibility of the newly developed method, validation was performed by including all analytes (malathion, malathion impurity-J, malathion mono isopropyl ester, malathion di isopropyl ester) according to international conference on harmonization (ICH) guidelines Q2(R1) [15] by considering the parameters like specificity, limit of detection, limit of quantification, linearity/range, accuracy/recovery, precision (system and method), robustness and ruggedness. Limit of detection and limit of quantification values were calculated by using slope and STYEX (n = 2), accuracy was calculated on % recovery (n = 3), precision was calculated on % RSD (relative standard deviation) (n = 5) and robustness and ruggedness were calculated on % RSD (n = 5).

RESULTS AND DISCUSSION

Optimization of chromatographic conditions: The results obtained from different trials carried out during the optimization of chromatographic conditions were given in Table-2.

Method validation

Specificity: The specificity of the method was performed by injecting blank, placebo sample, standard solutions of malathion, impurity-A, impurity-B, impurity-C, impurity-D, impurity-E, impurity-F, impurity-G, impurity-H, impurity-I, impurity-J, impurity-K, impurity-L, malathion mono isopropyl ester, malathion di isopropyl ester, α-terpineol, dipentene, pine needle oil and isopropyl alcohol and sample solution. All the impurities were spiked to sample solution. The chromatograms were verified for interferences from other impurities or the sample matrix.

Malathion mono isopropyl ester, malathion impurity-J and malathion di isopropyl ester were separated from each other compound. There was no interference of peaks from excipient, other impurities and blank peaks with malathion (RT- 34.9 min), malathion mono isopropyl ester (RT: 36.7 min), malathion impurity-J (RT: 37.6 min) and malathion di isopropyl ester (RT-38.3 min). The chromatogram containing the peaks for the impurities, excipients and sample was shown in Fig. 3.

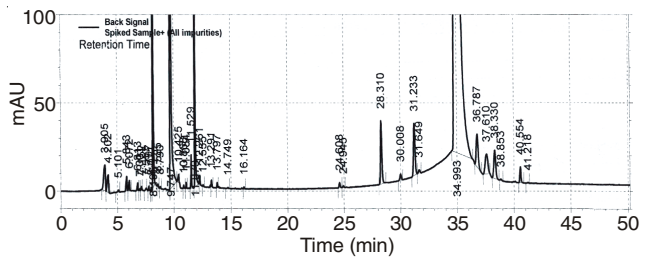


Fig. 3. Chromatogram containing the peaks for the impurities, excipients and sample

Limits of detection (LOD) and limit of quantification (LOQ): The LOD and LOQ of malathion mono isopropyl ester, malathion Impurity-J and malathion di isopropyl ester were estimated by residual deviation method (serial dilution). Standard solutions of 5, 10, 20, 30, 40 and 50 % were prepared and injected. Then, the SLOPE and STEYX were calculated by plotting the graph between concentration on (x-axis) and response on (y-axis). The values of LOD and LOQ were

TABLE-2
OPTIMIZATION OF CHROMATOGRAPHIC CONDITIONS

Trial No.	Column & specifications	Experimental conditions	Conclusions
1	DB-FFAP, 50 m × 0.32 mm × 0.25 µm	Initial oven at 100 °C, Injector at 103 °C Diluent: Isopropyl Alcohol	Impurities were co eluted
2	HP-5, 30 m × 0.32 mm × 0.25 µm	Initial oven at 100 °C, Injector at 103 °C. Individual impurity solutions injected Diluent: Isopropyl Alcohol	Malathion Impurity-J, Malathion Mono isopropyl ester, Malathion di isopropyl ester & Malathion were eluted at 8.7, 9.8, 10.1 and 9.5 min but with higher tailing. All the impurity peaks were very close to malathion peak.
3	HP-5, 30 m × 0.32 mm × 0.25 µm	Initial oven at 100 °C, Injector at 103 °C. Injected placebo and sample solution Diluent: Isopropyl Alcohol	In placebo solution peaks were identified at RT 8.1 to 10.4 and in sample solution impurity peaks were co-eluting with malathion peak
4	HP-5, 30 m × 0.32 mm × 0.25 µm	Initial oven at 70 °C, Injector at 103 °C. Injected placebo and sample solution Diluent: Isopropyl Alcohol	No improvement in separation of the impurity peaks from Malathion peak
5	HP-5, 30 m × 0.32 mm × 0.25 µm	Initial oven at 40 °C, Injector at 103 °C. Injected Impurity standard mix solution sample solution Diluent: Isopropyl Alcohol	No improvement in separation of the impurities
6	ZB-Multi residue-1 30 m × 0.25 mm × 0.25 µm	Initial oven at 100 °C, Injector at 150 °C. Injected Impurity standard mix solution sample solution Performed analysis by replacing the carrier gas "nitrogen" with hydrogen/helium Diluent: Isopropyl Alcohol	No improvement in separation of the impurities. Malathion peak was eluted with higher tailing
7	EC-5, 30 m × 0.32 mm × 1.0 µm	Initial oven at 60 °C, Injector at 170 °C. Injected impurity standard mix, individual impurity standards and sample solution Diluent: Isopropyl Alcohol	No improvement in separation of the impurities. Malathion peak was eluted with higher tailing
8	EC-5, 30 m × 0.32mm × 1.0 µm	Initial oven at 80 °C, Injector at 250 °C. Injected impurity standard mix, individual impurity standards and sample solution Diluent: Isopropyl alcohol	Malathion Impurity-J and Malathion di isopropyl ester were eluting at same retention time. Seperation was good when ethyl acetate was used as a diluent.
9	AT-1, 60 m × 0.25mm × 1.0 µm	Initial oven at 100 °C maintained for 2 min, then raised 5 °C/min to 230 °C, maintained for 20 min and then raised 20 °C/min to 280 °C maintained for 2 min Injector at 250 °C. Injected impurity standard mix, individual impurity standards and sample solution Diluent: Ethyl Acetate	Malathion mono isopropyl ester, Malathion Impurity-J and Malathion di isopropyl ester were separated from each other. No other peak interference was observed.

calculated from SLOPE and STYEX from the graph and given in Table-3.

$$LOD = STEYX \times 3.3/Slope$$

$$LOQ = STEYX \times 10/Slope$$

After establishing the LOD and LOQ concentrations, solutions at LOD and LOQ concentrations were prepared and injected. Limit of detection solution was injected in duplicate and peaks can be observed visually at LOD concentration. Limit of quantification Solution was injected in to six replicates and calculated the precision at LOQ level. The % RSD for malathion mono isopropyl ester, malathion impurity-J and malathion di isopropyl ester from six replicate injections of LOQ solution was 5.47, 9.79, 5.38 and 5.43 %, respectively.

Accuracy (% recovery): Accuracy of the method was evaluated by calculating the % recovery of malathion mono isopropyl ester, malathion impurity-J and malathion di isopropyl ester at three different concentrations of LOQ, 100 % and 150 % of specification level. Each level has been analyzed in triplicate. The recovery of all these substances was found to be in between the predefined acceptance criteria of 85 to 115 %. The obtained recovery for malathion mono isopropyl ester was between 99.4 to 106.4 %, for malathion impurity-J was between 104.2 to 113.5 % and for malathion di isopropyl ester was between 97.4 to 114.1 %. Hence it was concluded that the method was found to be accurate.

Linearity and range: The linearity was established by injecting the malathion mono isopropyl ester, malathion

TABLE-3
RESULTS OF LOD, LOQ AND RANGE OF MALATHION MONO ISOPROPYL ESTER, MALATHION IMPURITY-J AND MALATHION DI ISOPROPYL ESTER

Name of the impurity	LOD (%)	LOQ (%)	Range (%)
Malathion mono isopropyl ester	0.004	0.012	0.012 to 0.30 (LOQ to 150 %)
Malathion impurity-J	0.005	0.016	0.016 to 0.30 (LOQ to 150 %)
Malathion di isopropyl ester	0.003	0.009	0.009 to 0.30 (LOQ to 150 %)

impurity-J and malathion di isopropyl ester at 6 different concentrations ranging from LOQ to 150 % (LOQ, 50, 75, 100, 125 and 150 %) of specification level. 100 % concentration was equivalent to 0.20 % (specification limit) with respect to sample concentration. The linearity graphs for malathion mono isopropyl ester, malathion impurity-J and malathion di isopropyl ester were given in Fig. 4. The correlation coefficient for malathion mono isopropyl ester was 0.996, malathion impurity-J was 0.995 and for malathion di isopropyl ester was 0.996 against the acceptance criteria of NLT 0.99. Hence, it was concluded that the method was linear. The range was established based on the linearity and accuracy results and was given in Table-3.

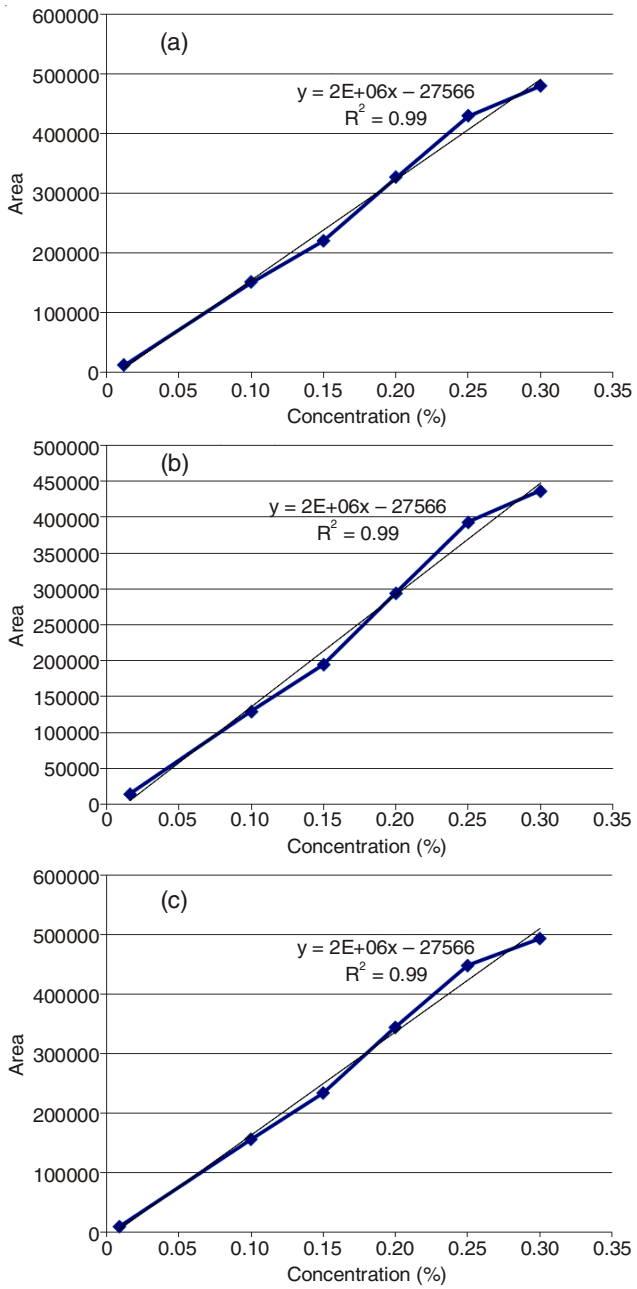


Fig. 4. Linearity graphs of malathion mono isopropyl ester impurity (a), malathion impurity J (b) and malathion di isopropyl ester (c)

Precision: The precision of the method was evaluated for system precision and method precision.

(a) System precision: The system precision was evaluated by analyzing six replicate injections of standard solution. The % RSD for area response was determined to assess the system precision. The % RSD for the area of malathion mono isopropyl ester, malathion impurity-J and malathion di isopropyl ester was 4.1, 4.1 and 4.1 % respectively which was within the acceptance criteria of NMT 15 % for each compound. Hence the system was precise.

(b) Method precision: The method precision was evaluated by analyzing six replicate preparations of spiked sample and the content of malathion mono isopropyl ester, malathion impurity-J and malathion di isopropyl ester was calculated. The % RSD for the content of malathion mono isopropyl ester, malathion impurity-J and malathion di isopropyl ester in method precision study are 6.4, 6.1 and 6.2 %, respectively, which was within the acceptance criteria of NMT 15 %. Hence the method was precise.

Ruggedness

(a) Day to day reproducibility: The reproducibility was established by performing the analysis for determination of malathion mono isopropyl ester, malathion impurity-J and malathion di isopropyl ester contents on two different days. The combined % RSD (method precision and day-2) for the content of malathion mono isopropyl ester was 11 %, for malathion impurity-J was 7.1 % and for malathion di isopropyl ester was 12.2 %, which were within the acceptance criteria of NMT 15 % for each compound.

(b) Intermediate precision (change in column and analyst): The intermediate precision was established by changing the column and analyst. The combined % RSD (method precision and Analyst-2) for the content of malathion mono isopropyl ester was 11.7 %, for malathion impurity-J was 14.3 % and for malathion di isopropyl ester was 13.5 %, which were within the acceptance criteria of NMT 15 % for each compound.

(c) Solution stability: The stability of the analytical solution was evaluated by injecting the standard solution and sample solution at a regular intervals of initial (0), 6, 12, 18 and 24 h along with the placebo solution.

The % RSD for areas of standard solution from initial to after 24 h for malathion mono isopropyl ester was 7.4 %, for malathion impurity-J was 7.7 % and for malathion di isopropyl ester was 7.4 % are within the acceptance criteria of NMT 15 % for each compound. Hence the standard solution was stable up to 24 h.

Robustness: The robustness of the method was studied by deliberately changing the nitrogen gas pressure and the GC oven temperature. The effect of nitrogen gas pressure was studied at 33 and 40 psi (optimized pressure was 36.4 psi). The effect of GC oven temperature was studied at 119 °C and 121 °C (optimized temperature was 120 °C). The % RSDs of peak area for malathion mono isopropyl ester, malathion impurity-J and malathion di isopropyl ester along with initial method precision data were given in Table-4. Hence it was concluded that the method was robust even after deliberate changes in the nitrogen gas pressure and the GC oven temperature.

Forced degradation: Forced degradation of malathion lotion 0.5 % w/v USP was carried out, to confirm that during

TABLE-4
ROBUSTNESS DATA

Parameter	Variation	% RSD of the Area		
		Malathion mono isopropyl ester	Malathion Impurity-J	Malathion di isopropyl ester
Method precision	36.4 psi and 120 °C	4.10	4.10	4.10
Pressure of the nitrogen gas	33 psi (low)	2.69	2.81	2.69
	40 psi (high)	3.53	3.61	3.49
GC oven temperature	119 °C (low)	9.79	9.88	9.78
	121 °C (high)	5.33	5.53	5.56

TABLE-5
RESULTS OF THE FORCED DEGRADATION STUDIES

Type of degradant	Area (%)			
	Malathion	Malathion mono isopropyl ester	Malathion impurity-J	Malathion di isopropyl ester
Sample as such	94.42	0.02	0.06	Not detected
Acid degradation: (with 0.1 N HCl heat at 50 °C up to 5 h)	94.99	0.35	0.04	Not detected
Acid degradation: (with 1 N HCl heat at 50 °C up to 10 h)	82.72	10.45	Not detected	Not detected
Base degradation: (with 0.1 N NaOH heat at 50 °C up to 5 h)	21.32	0.40	0.21	Not detected
Base degradation: (with 0.5 N NaOH heat at 50 °C up to 5 h)	Not detected	Not detected	0.05	Not detected
Oxidative degradation: (with 3.0 % H_2O_2 heat at 50 °C up to 5 h)	92.40	0.02	0.07	Not detected
Oxidative degradation: (with 3.0 % H_2O_2 heat at 50 °C up to 10 h)	93.21	0.04	0.06	0.03
Thermal degradation: (at oven temp 50 °C up to 5 h)	93.68	0.04	0.03	Not detected
Photolytic degradation: (sample kept 92 h under UV chamber)	95.17	Not detected	Not detected	Not detected

stability study or throughout the shelf life, any degradation product if found will not interfere with the malathion mono isopropyl ester, malathion di isopropyl ester and malathion impurity-J peaks. In addition, the forced degradation study would help to identify the type of degradation pathway (whether oxidative, alkali hydrolysis, acid hydrolysis, photolytic *etc*.) for each of the degradant.

Forced degradation was performed by degrading the sample with 0.1 N HCl, 1 N HCl, 0.1 N NaOH, 0.5 N NaOH, 3 % hydrogen peroxide, thermal heating at 50 °C and photolytic degradation. From the forced degradation studies, degradation was not observed in oxidative degradation, photolytic degradation and thermal degradation. However, degradation was observed in acid degradation and base degradation. The results were given in Table-5.

Conclusion

The proposed method was a new GC-NPD method for the determination of impurities in malathion lotion. The method was fully validated according to the ICH guidelines and presented good linearity, specificity, accuracy, precision and robustness and it was also found to be simple, sensitive, selective and stability indicating. The LOD and LOQ values were established by using Slope and STYEX method. The proposed method can be successfully applied for the determination of the impurities in Malathion lotion. The developed method using gas chromatographic technique is not only useful for malathion lotion, but also may be useful for estimation of other organophosphorous compounds.

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