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Stability indicating nature of RP-HPLC method for determination of impurity profile in malathion

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ABSTRACT

The objective of the present work was to develop stability indicating RP-HPLC method for determination of impurities in Malathion on YMC-Pack ODS AQ 250 X 4.6mm 5µm column using a gradient mixture of solvent A (Milli Q water, adjust the pH of the above solution to 2.5 using Orthophosphoric acid) and solvent B (Methanol). The flow rate is 0.7ml/min and the detection wavelength is 210nm. Statistical analysis proved the method to repeatable, specific and accurate for estimation of Malathion and its impurities. It can be used as a stability indicating method due to its effective separation of the drug from its impurities.

Keywords: RP-HPLC, Malathion, Stability, Validation.

INTRODUCTION

The international conference on harmonization (ICH) guide-lines [1-3] emphasizes that the purity of drug susceptible to change during storage, must be determined by using validated stability- indicating methods, which can selectively determine the drug in presence of its related impurities. Malathion chemically Known as diethyl (dimethoxy thiophosphorylthio) succinate[4], has an empirical formula of $C_{10}H_{19}O_6PS_2$ and a molecular weight of 330.358 [Fig-1]. Malathion is an organophosphate parasympathomimetic which binds irreversibly to cholinesterase. Malathion is an insecticide of relatively low human toxicity[5]. In the former USSR, it was known as carbophos, in New Zealand and Australia as maldison and in South Africa as mercaptothion.

Malathion is an insecticide of relatively low human toxicity. Malathion is a pesticide that is widely used in agriculture, residential landscaping, public recreation areas, and in public health pest control programs such as mosquito eradication[6]. Today, Winnipeg is the only major city in Canada with an ongoing Malathion nuisance-adult-mosquito-control program[7,8]. In the US, it is the most commonly used organophosphate insecticide[9]. Malathion in low doses (0.5% preparations) is used as a treatment for Head lice and body lice[c]. Malathion is approved by the United States Food and Drug Administration for treatment of pediculosis[10,11]. It is claimed to effectively kill both the eggs and the adult lice, but in fact has been shown in UK studies to be only 36% effective on head lice, and less so on their eggs[12]. This low efficiency was found when malathion was applied to lice found on schoolchildren in the Bristol area in the UK and it is assumed to be caused by the lice having developed resistance against malathion. Malathion itself is of low toxicity; however, absorption or ingestion into the human body readily results in its metabolism to malaoxon[13,14], which is substantially more toxic[15]. In studies of the effects of long-

term exposure to oral ingestion of malaoxon in rats, malaoxon has been shown to be 61 times more toxic than malathion[15]. It is cleared from the body quickly, in three to five days [16]. According to the United States Environmental Protection Agency there is currently no reliable information on adverse health effects of chronic exposure to malathion[17].

Besides the reported impurities, we observed Impurity-A chemically known as O,O,S-Trimethyl phosphorodithioate, Impurity-B chemically known as O,S,S-Trimethyl phosphorodithioate, Impurity-C chemically known as Trimethyl phosphorothioate, Impurity-D chemically known as Isomalathion, Impurity-E chemically known as Malaoxon, Impurity-F chemically known as Dimethyl malathion, Impurity-G chemically known as Methyl malathion, Impurity-H chemically known as Diethyl fumarate, Impurity-I chemically known as O,O-dimethyl-S-(1-carboxy-2-carboethoxy) ethyl phosphorodithioate, Impurity-J chemically known as O,O-methyl ethyl S-(1,2-dicarboethoxy)ethyl phosphorodithioate, Impurity-K chemically known as Diethyl 2-mercaptosuccinate, Impurity-L chemically known as Tetraethyl Dithiosuccinate and Diethyl maleate Hence, a stability-indicating RP-HPLC method for determination of Malathion and impurities was developed and validated as per international conference on harmonization (ICH) guidelines.

MATERIALS AND METHODS

All reagents were obtained commercially and were of the highest commercial quality and used without further purification. Solvents were freshly distilled and used. TLC or HPLC routinely checked the purity of all compounds. IR spectra were recorded on a Perkin-Elmer model spectrum100 instrument. 1H-NMR (400 MHz) and 13C-NMR (100MHz) spectra were recorded in $CDCl_3$ or DMSO using Brucker instrument and Mass spectra were recorded on a Perkin-Elmer mass spectrometer operating at 70 eV.

Chemicals and reagents

Malathion and its impurities viz. O,O,S-Trimethyl phosphorodithioate, O,S,S-Trimethyl phosphorodithioate, Trimethyl phosphorothioate, Isomalathion, Malaoxon, Impurity-F Dimethyl malathion, Methyl malathion, Impurity-H Diethyl fumarate, Impurity-I O,O-dimethyl-S-(1-carboxy-2-carboethoxy) ethyl phosphorodithioate, O,O-methyl ethyl S-(1,2-dicarboethoxy)ethyl phosphorodithioate, Diethyl 2-mercaptosuccinate, Tetraethyl Dithiosuccinate and Diethyl maleate were obtained from Suven Life sciences Ltd. Orthophosphoric acid, Methanol, Acetonitrile, hydrochloric acid, sodium hydroxide, hydrogen peroxide (30%), sodium chloride and barium chloride were obtained from Rankem, New Delhi, India. All solution are prepared in Milli Q water (Millipore USA)

HPLC instrumentation and conditions.

Waters Alliance 2695 separation module (Waters Corporation, Milford, USA) equipped with 2996 PDA detector (for specificity and forced degradation studies) and 2487 UV/visible detector with Empower software was used for the analysis. YMC-Pack ODS AQ column (250 X 4.6 mm, 5um YMC Corporation, Japan) and a gradient mixture of solvent A and B were used as stationary and mobile phases, respectively. Buffer contains 1000mL of water; adjust the pH to 2.5 using Orthophosphoric acid. Buffer was used as solvent A. Methanol was used as solvent B. The gradient program (T/%B) was set as 0/55, 15/55, 20/50, 40/75, 50/75, 50.1/55 and 60/55. 0.7ml/min flow rate and 20µL of injection volume were maintained. The eluted compounds were monitored at 210nm. The column oven temperature was maintained at 25°C.

Preparation of standard solutions

Acetonitrile was used as diluent. 0.2mg/ml solution of Malathion, Impurity-H and Impurity-I was prepared in diluent for system suitability. A blend of six 0.10% Malathion impurities was prepared in diluent with respect to 20.0mg/ml of Malathion

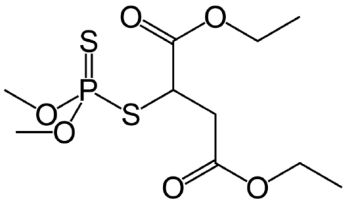

Fig-1: Structure of Malathion.

HPLC method development

The HPLC method was optimized so as to obtain a stability- indicating method that can resolve impurities from Malathion. The buffer having pH 2.5 was adopted, because, it was suitable to separate the impurities from Malathion. YMC-Pack ODS AQ (250 X 4.6 mm, 5um) column allowed to a rapid resolution between all the impurities and showed the best values of theoretical plates and symmetry for Malathion.

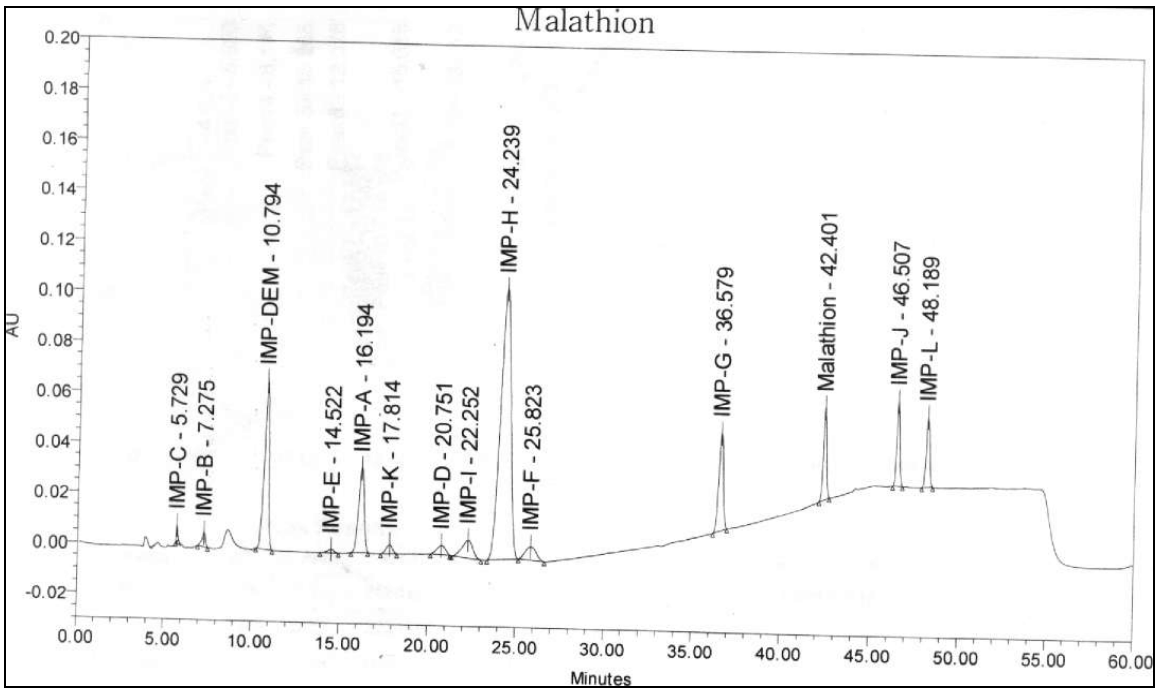

Fig-2: A typical chromatogram of Malathion spiked with 0.10% of impurities.

Method Validation

The developed method was validated as per ICH guidelines and the results are given (Table-I). The specificity of the developed HPLC method for Malathion was determined in the presence of its process impurities. All the analysis was carried out by HPLC with PDA detector. The chromatographic peak purity tool, applied to Malathion and its impurities peaks, demonstrated that all the peaks were pure in all cases conform the absence of other impurities co-eluting in the same retention time and there by signifying the specificity and stability indicating nature of the method. The detection limit (DL) and quantification limit (QL) for all impurities were determined at a signal to noise ratio of 3:3 and 10:1 respectively, by using Empower software. Precision study was carried at QL level by injecting six times and calculating the percentage of R.S.D of area of all impurities. Linearity test solutions for six concentration levels from QL to 150 % of the specification level (0.10%). Peak area versus concentration data was performed by least-squares linear regression analysis. Standard addition and recovery experiments were conducted to determine accuracy of impurities quantification in bulk drug samples. The study was carried out in triplicate at QL, 100% and 150% level with respect to specification 0.1%. The percentages of recoveries for impurities were calculated. The robustness of developed method was determined by altering experimental conditions purposely and evaluating the resolution between Malathion and all impurities. Flow rate was changed by $\pm$ 0.1 units, all above varied conditions the components of the mobile phase were held constant and no significant change (relative error less than 5%) of relative retention time was observed. Significant changes were not observed in all the impurities. The stability data confirmed that sample solutions were stable up to 48hrs. The system suitability was established in terms of resolution between Impurity-H and Impurity-I which was more than 1.0, when a 20.0mg/ml Malathion solution spiked with 0.10% of all impurities were injected.

Table-I Validation data of the developed method

Parameter	IMP- A	IMP- B	IMP- C	IMP- D	IMP- E	IMP- F	IMP- G
DL(%)	0.004	0.020	0.040	0.030	0.040	0.020	0.003
QL(%)	0.020	0.030	0.050	0.050	0.050	0.030	0.010
Method Precision(%RSD)#	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Intermediate Precision(%RSD)#	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Accuracy(%recovery) at:-							
QL(%)	100.740	105.180	100.990	86.110	107.530	104.390	102.270
100%	101.090	99.880	107.120	97.990	97.950	107.540	99.490
150%	101.680	100.790	100.480	100.850	101.630	102.450	100.810

Parameter	IMP- H	IMP- I	IMP- J	IMP- K	IMP- L	IMP- DEM
DL(%)	0.001	0.020	0.002	0.020	0.004	0.001
QL(%)	0.100	0.030	0.010	0.030	0.010	0.010
Method Precision(%RSD)#	0.000	0.000	0.000	0.000	0.000	0.000
Intermediate Precision(%RSD)#	0.000	0.000	0.000	0.000	0.000	0.000
Accuracy[a](%recovery) at:-						
QL(%)	91.130	102.500	99.840	100.650	101.740	101.150
100%	99.080	101.410	98.560	100.080	99.940	99.070
150%	97.660	107.030	99.610	100.310	100.160	100.260

[a] Carried at QL, 100% and 150% level with respect to specification (0.10%)

Forced degradation studies result

The stability -indicting ability of the developed method was studied by conducting forced degradation studies on Malathion. Forced degradation samples were injected at regular intervals and the final stress conditions were established so as to obtain 10-30% degradation of Malathion. Related substances by HPLC method shown degradation in oxidative degradation, acid degradation, thermal degradation, base degradation and water degradation, no degradation observed in photolytic degradation.

CONCLUSION

A stability indicating HPLC method has been developed and validated for the purity determination of Malathion. The behavior of Malathion under various stress conditions was studied. This method is able to separate the Malathion from its degradation impurities; it can be conveniently applied for the testing of batch release and stability studies (Table -II).

Table II: Summary of forced degradation studies

Stress type	IMP- A	IMP- B	IMP- C	IMP- D	IMP- E	IMP- F	IMP- G
Unstressed	BQL	BDL	BDL	BQL	BDL	BDL	0.01
Acid degradation	0.04	0.31	0.23	0.07	BDL	BDL	0.01
Base degradation	0.55	0.15	0.74	BDL	0.16	BDL	0.01
Photolytic degradation	BQL	BDL	BDL	BQL	BDL	BDL	BQL
Oxidative degradation	BQL	0.08	BDL	0.06	BDL	BDL	0.01
Humidity degradation	0.12	BDL	0.15	0.10	BDL	BDL	0.01
Thermal degradation	0.42	5.08	0.47	13.12	15.69	BDL	0.18

Parameter	IMP- H	IMP- I	IMP- J	IMP- K	IMP- L	IMP- DEM	MUI	TI
Unstressed	BDL	BDL	0.02	BDL	0.05	BDL	0.03	0.17
Acid degradation	BDL	7.30	0.02	1.58	0.05	BDL	1.41	12.62
Base degradation	0.31	1.33	0.02	1.83	0.05	0.07	11.74	31.84
Photolytic degradation	BDL	BDL	0.04	BDL	0.05	BDL	0.02	0.20
Oxidative degradation	BQL	0.56	0.03	0.06	0.47	BQL	0.13	2.42
Humidity degradation	BDL	BDL	0.03	0.11	0.06	BDL	0.51	1.07
Thermal degradation	5.16	BDL	4.11	7.84	3.25	0.77	6.83	64.04

MUI Major unknown impurity, TI: Total impurities, BDL: Below detection limit, BQL: Below Quantification limit

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