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Development and validation of a headspace gas chromatography mass spectrometry method for determination of residual solvents in five Triazole fungicides technical.

Chemistry

KEYWORDS: Residual solvents, triazole fungicides, HS-GC-MS, SIM mode, Method development, Method validation.

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ABSTRACT

A simple and sensitive method for the simultaneous determination of residual solvents, viz.:-cyclohexane, toluene, dimethylformamide (DMF) and 1,4-dichlorobenzene in five triazole fungicide technical by headspace technique with mass detection is described. The linear regression analysis data for the calibration curve for all monomers showed a good linear relationship with a regression coefficient of 0.99 over the concentration. The limit of detection and limit of quantification for the target analytes were in the range from 0.018 to 6.1 mg/kg and 0.05 to 1.6 mg/kg respectively.

Introduction:

Residual solvents are Organic Volatile Impurities (OVI's) that are used or produced in the manufacturing of technical substance. Residual solvents refer to the amount not removed during the purification of the final product. They may be identified or unidentified, volatile or non-volatile; include starting materials, by-products, intermediates, degradation products, reagents, ligands and catalysts. If production or purification process involves the use of such solvents the product should then be tested for the residual solvents. A cumulative method may be used to calculate the residual solvent levels in the product on the basis of their levels in the ingredients that are used to produce the final product. Exposure limits in guideline are established by referring to methodologies and toxicity data described in Environmental Health Criteria (EHC) and the Integrated Risk Information system (IRIS) monographs. However, some specific assumptions about residual solvents to be used in the synthesis and formulation of products should be taken into account in establishing exposure limits. Residual solvents are not desirable substances in the final product so their acceptable limits have been published in ICH guidelines[1]. In IARC[2] guidelines also solvents are classified into different classes according to their toxicity. Methods are developed and validated for pharmaceutical products[3-4] using headspace gas chromatograph FID. Although there is no specific limits of solvents in pesticides as in pharmaceuticals, pesticide regulatory authorities have started asking for the same. Hence, in pesticides not much method development has taken place. In the present work, a simple and sensitive method has been developed for the determination of residual solvents in hexaconazole, propiconazole, tebuconazole, difenconazole and myclobutanil fungicides technical[5] grade samples using headspace gas chromatograph mass spectrometer (HS-GC-MS).

The separation was achieved on DB-624 column (30 m x 1.8 mm x 0.32 µ) in 26 minutes for the commonly used solvents viz.:-cyclohexane, toluene, dimethylformamide (DMF) and 1,4-dichlorobenzene and the analysis was done in SIM mode to enhance detection limit at 120 °C for 20 minutes. The developed method was validated according to SANCO[6] guideline to evaluate the capability of the method.

Experimental:-

1.1 Material:- Toluene, Dimethyl formamide, cyclohexane and 1,4-dichlorobenzene of AR grade were purchased form Merck. Hexaconazole, Propiconazole, Tebuconazole, Difenconazole and Myclobutanil technical samples were taken from Indofil Industries Limited.

1.2 Method: - All measurements were carried out using an Agilent 7890 GC, automatic 7697A Headspace and 7000 Triple Quad MS. GC conditions were optimized using DB-624 (30 m x 1.8 mm x 0.32 µ) capillary column using helium as a carrier gas at a flow rate of 1.2 mL/minute at 40 °C initially with 10 minutes hold time and then increasing the temperature at rate of 30 °C per minute upto 240 °C and hold for 5 minutes hold. Injection was done on split mode with 5:1 split ratio at 240 °C of injector temperature. Headspace operating conditions were: 20 minutes for sample equilibration at a temperature of 120 °C, transfer line temperature 180 °C, vial pressurization time of 0.2 minutes, sample loop fill time of 0.3 minutes, and loop equilibration time of 0.2 minutes and injection time of 0.5 minutes. The detection was done on mass spectrometer using SIM mode for 56, 84 ions for cyclohexane, 44.1, 73.1 ions for dimethyl formamide, 65, 91 for toluene, 75, 110.9, 145.9 ions for 1,4-dichlorobenzene all four solvents.

1.2.1Preparation of solutions:

1.2.1.1Solvent Standard: - A quantity of about 50 mg of cyclohexane, toluene, 1,4-dichlorobenzene and 125 mg of dimethylformamide standards were weighed into a 25 mL volumetric flask and diluted upto the volume with dimethyl sulphoxide (Solution-A). Pipetted 1.0 mL from the solution-A into 100 mL volumetric flask and diluted upto the volume with dimethyl sulphoxide (Solution-B). 1.0 mL from solution-B and 1.0 mL of dimethyl sulphoxide was added into 20 mL head space vial and subjected to analysis by head space gas chromatography mass spectrometer (SIM mode).

1.2.1.2Preparation of technical sample: A quantity of 100 mg of triazole technical sample was weighed and transferred into a 20 mL head space vial. 2.0 mL of dimethyl sulphoxide was pipetted into 20 mL head space vial subjected to analysis by head space gas chromatography mass spectrometer (SIM mode).

2. Results and Discussion:

2.1 Methodology:-

To ensure maximum mass transfer of all solvents distributed in solid and liquid phase into vapor phase in sealed headspace vial critical parameters were optimized such as vial equilibration temperature, vial equilibration time and sample quantity.

2.1.1Evaluation of the completeness of mass transfer:-

To check for completeness of solute mass transfer from solid and condensed phases to the vapor phase and for the efficiency of headspace extraction the sample vial was purged with fresh helium after each run to remove the residual vapor from the previous headspace extraction. The peak areas, A1 and A2, represent the vapor concentration of solvent standards from the first and second headspace extraction, respectively. The ratio (R), i.e., A2/A1, is regarded as a parameter for indicating the completeness of the headspace extraction. The smaller value of R, the more complete the solute mass transfer.

2.1.2Vial equilibration temperature: -
Mass transfer of solvent standards was checked by applying temperature to the head space vial at constant time (30 minutes) so as to increase transfer rate and to have maximum mass transfer in minimum time. The maximum mass transfer was observed at 120 °C temperature of vial which is shown in figure-01.

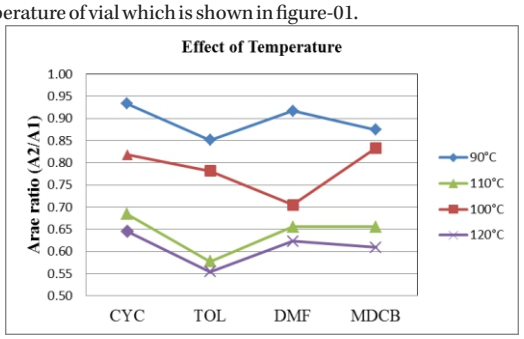

Figure-01 Effect of vial equilibrium temperature

2.1.3 Vial equilibration time: -
For an efficient sample analysis, rapid and near-complete mass transfer is highly desired. So the effect of time was checked for the maximum output at 5.0, 10.0, 20.0 and 30.0 minutes**.** Figure-02 shows that a near-complete mass transfer in headspace vial can be achieved within 20.0 minutes at 120 °C temperature.

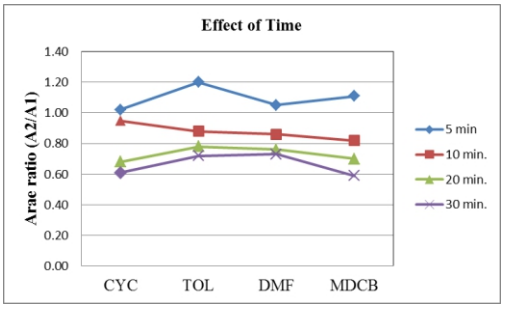

Figure-02 Effect of vial equilibration time

2.1.4 Sample quantity: - As the headspace extraction is performed in a closed vial, and the quantification will be done at low level; sample size need to be optimized to have consistency in results obtained. An acceptable response in working linear range was with 50-150 mg of sample quantity.

Therefore the optimized condition for residual solvent analysis was 100 mg of sample heated in a sealed headspace vial at 120 °C for 20 minute.

2.2 Method Validation: - Validation of the developed method was done for following parameters:

2.2.1System Suitability: - System suitability was evaluated for number of theoretical plates and tailing factor by injecting six replications of standard solution for each solvent standard. Theoretical plate observed was more than 2000 and tailing factor was less than 2.0 for all solvent standards, both meeting the acceptance criteria of system suitability.

2.2.2 Specificity/Selectivity: -
Specificity of the method was checked for any interference from blank and from each other (solvent standard). It was observed that there is no interference at the retention time of the solvent standards due to the blank. Figure-03 and figure-04 showed that there is no interference at the retention times of standards. Therefore, the specificity of the method was judged by the absence of any interference at the analyte elution times from blank chromatograms and from each other. All standards can be identified on the basis of their retention time i.e. retention time for cyclohexane is 5.039 minutes, for toluene is 7.961 minutes, for dimethyl formamide is 9.685 minutes and for 1,4-dichlorobenzene is 13.180. Since, the analysis was carried out in SIM mode, the method is selective. The selectivity of the method was determined by using selective ions (44.1, 56, 65, 73.1, 75, 84, 91.0, 110.9, and 145.9) which was not observed in distilled water as blank solution.

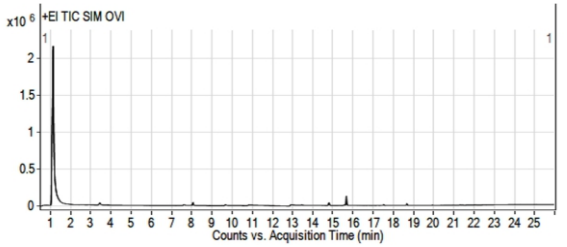

Figure-03 – SIM chromatogram for blank

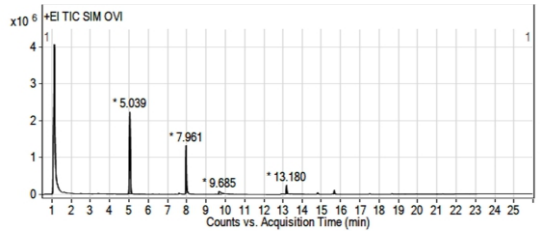

Figure-04 – SIM chromatogram for Solvent Standards.

2.2.3 Linearity: The Linearity of detector response was demonstrated over the different concentration range of each solvent standard. Regression coefficient found was more than 0.99 which shows that the detector response was linear over the specified concentration range for each of solvent standard and tabulated in table-01.

2.2.4 Limit of detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ for each found were tabulated in table-01 of each solvent standard in mg/kg. The % RSD of peak areas at limit of quantification level for seven replications was well within the acceptance limit.

Sr. No	Solvent Standards	Concentration (mg/kg)	Regression coefficient (r^2)	LOD mg/kg	LOQ mg/kg
1	Cyclohexane	10.28 to 1028	0.995	0.018	0.05
2	Toluene	10.64 to 1064	0.997	0.032	0.08
3	Dimethylformamide	26.45 to 2116	0.995	6.1	1.6
4	1,4-dichlorobenzene	24.55 to 2056	0.996	0.18	0.5

Table-01: Linearity, LOD and LOQ data for solvent standards

2.2.5 Precision: The precision in the conditions of repeatability (for seven sample preparations in a single day) was determined for Tebuconazole, Difenconazole and Propiconazole technical samples in each replicate of test item. The % RSD found for 0.005 %, w/w of cyclohexane was found to be 3.5, 0.0002 %, w/w of toluene was found to be 5.7 in Tebuconazole. The % RSD found for 0.0005 %, w/w of toluene was found to be 6.2, 0.009 %, w/w of dimethyl formamide was found to be 3.6 in Difenconazole. The % RSD found for 0.0002 %, w/w of toluene was found to be 5.9, 0.002 %, w/w of 1,4-dichlorobenzene was found to be 2.9 in Propiconazole. Results obtained were well within the calculated %RSD as per Horwitz equation.

2.2.6 Accuracy: - To confirm the accuracy of the proposed method, recovery experiments were carried out by the standard addition technique. The accuracy of the method was carried out by fortifying solvent standards in technical grade triazole sample at LOQ and 10 x LOQ levels (Five sample preparations at each level). The mean percentage recoveries found were within the acceptable recoveries of 80-120 % at both levels.

2.2.7 Sample Results: - Residual solvent content, expressed as

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mg/Kg, in different triazole fungicide samples were analyzed in duplicate using the proposed procedure (table 02).

Product	Content of residual solvents, mg/kg			
	Cyclohexane	Toluene	Dimethylformamide	1,4-dichlorobenzene
Hexaconazole	NA	1.44	ND	NA
Tebuconazole	48.6	1.8	ND	NA
Propiconazole	NA	2.4	NA	19.7
Difenconazole	NA	5.2	88.2	NA
Myclobutanil	NA	111.6	ND	NA

NA-Not applicable, **ND**-Not detected
Table-02:- Residual solvent content results in triazole fungicide technical samples.

3. Conclusion: The validation results clearly show that this method is suitable for the quantification of free (residual) solvents at low level in Hexaconazole, Tebuconazole, Propiconazole, Tebuconazole and Myclobutanil technical grade fungicides. A simple, fast and precise method was successfully developed and validated for the estimation of residual solvents by HS-GC-MS. Validated method is useful for monitoring and controlling the process.

4. Abbreviations
HS-GC-MS - Head space Gas chromatography mass spectrometry
MS - Mass spectrometer
SIM- Selective ion monitoring
RT- Retention time
STD DEV- Standard deviation
% RSD- Percentage Relative standard deviation

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