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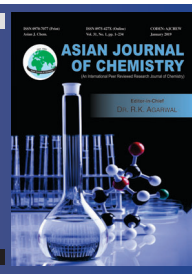

Development and Validation of Headspace GC-FID Method for Simultaneous Determination of Residual Solvents in Ethyl-3-methyl-3-phenyl Glycidate

CHANDRAKANT V. ZURE and RAHUL V. PINJARI*

School of Chemical Sciences, Swami Ramanand Teerth Marathwada University, Nanded-431606, India

*Corresponding author: E-mail: rvp.chem@srtmun.ac.in

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Ethyl-3-methyl-3-phenylglycidate is being used in flavor, fragrance and pharmaceutical industries. Generally acetonitrile, benzene, toluene, methanol and dichloromethane are used as solvent in synthesis of ethyl-3-methyl-3-phenylglycidate. Complete removal of such organic volatile components is not always possible and they may remain at residual level. However, these solvents are harmful and toxic to human as well as in environment above certain concentration. This necessitates the identification of the residual solvents and controlling them within residual solvents acceptance limit set by ICH (Q3C) guidelines. In present work, simple and rapid gas chromatographic method using head space sampler and flame ionization detector have been developed for determination of a residual solvents in ethyl-3-methyl-3-phenylglycidate. This method resulted into a well separation of residual solvents on non-polar capillary columns. The method is validated for precision, recovery, linearity, range and limit of detection as per ICH guideline.

Keywords: Ethyl-3-methyl-3-phenylglycidate, Flavour, Head space Gas chromatography, Residual solvent.

INTRODUCTION

Organic solvents (volatile substance) are routinely used for the synthesis of pharmaceutical, drugs, food and flavouring agent. However presence of these solvents is not desirable in such products, since they are harmful, toxic and hazardous to human being as well as environment [1]. These residual solvents may remain in the final product due to either incomplete removal or generated as a degradant byproduct. Pharmaceutical, food and flavour ingredient manufacturers always have concern regarding the usage of organic volatiles and emphasize on avoiding manufacturing process with harmful solvents. However, yield of product and crystallization process to purify the product are highly dependent on the solvent systems used [2]. Therefore, the usage of the undesired solvents in synthetic procedure cannot be avoided in most of the synthetic procedures. The residual solvents can modify the properties of certain compounds and also adversely affect their physico-chemical properties *viz*. colour, odour, solubility, *etc*. [3]. Thus special importance is given to residual solvents analysis in food and pharmaceutical industries. The solvents carried forward to final product could often come in contact with human/environment and causes harm [4]. Therefore, International Council for Harmonization (ICH) has evaluated possible risk of different residual solvents and subsequently classified them in to three classes with specific acceptable limit [5]. Class-1 solvents (*e.g*. benzene, carbon tetrachloride, 1,2-dichloroethane) possess unacceptable toxicity and carcinogenicity and hence they should be avoided in the production of drug substances and excipients. The Class-2 solvents like methanol, acetonitrile, dichloromethane are less toxic and they can be used in the production, however their concentration in the final product is limited to definite concentration levels. The residual solvents falling in Class-3 *viz*. ethanol, ethyl acetate, dimethyl sulfoxide are least toxic to human and environment and their use is preferred over Class-1 and 2 solvents. Ethyl-3-methyl-3-phenylglycidate (EMPG) is used in flavours and precursor of pharmaceutical products [6,7]. It has colourless to pale yellow colour and sweet strawberry like odour. Ethyl-3-methyl-3-phenylglycidate is added in hard candies, pepper-mints, cherry-flavoured lollipops and butterscotch, *etc*. to impart strawberry flavour [8]. Ethyl-3-methyl-3-phenylglycidate is primarily synthesized from

acetophenone and chloroethylacetate in presence of different solvents *viz*. methanol, acetonitrile, dichloromethane, benzene and toluene [9-13]. The ICH has set the concentration limit for these solvents in final product to 3000, 410, 600, 2 and 890 ppm, respectively. Acetonitrile is more preferable solvent for getting high yield [9,10], whereas diastereoselectivity of EMPG in MeOH is very poor [7]. During the synthesis of EMPG, ethanol and ethyl acetate are generated, however being Class-3 solvents they will not be considered for residual solvent analysis in the present investigations.

Analytical method for separation and quantification of E/Z-EMPG using HPLC has been reported in the literature [13]. The run time of this method is 60 min, *n*-hexane/2-propanol and Lux Cellulose-4 column is used. However, the method for determination of residual solvents in EMPG having two chiral carbons is not available in the literature. Furthermore, the gas chromatography (GC) is more sensitive for residual solvent analysis in organic compound than the HPLC. The headspace (HS) GC analysis is the best choice for separation and quantification of residual solvents. HS-GC technique primarily depends on the liquid-vapour phase equilibrium and all of the components in a sample may not evolve into headspace gas volume. Since the organic solvents are relatively volatile and have high vapour pressure, they easily reflect in the headspace gas volume. HS-GC has ability to quantify individual solvents accurately and it is more robust than direct liquid injections methods [14]. HS-GC applied for residual solvents in pharmaceuticals, food and packing material [15]. Thus, HS-GC method development and validation for residual solvents in the EMPG have been presented in this article.

EXPERIMENTAL

Analytical grade methanol, acetonitrile, benzene, HPLC grade toluene and dichloromethane are purchased from Fisher Scientific India, while HPLC grade dimethyl sulfoxide is purchased from Rankem. Ethyl-3-methyl-3-phenylglycidate (EMPG) is purchased from Sigma Aldrich.

Instrumentation: Agilent 7890BGC equipped with flame ionization detector, split/split less injection port, headspace sampler 7697A for loading samples, 0.53 mm fused silica transfer line is used. A Shimazdu analytical balance D432613343 and Thermoscientific single channel auto-pipettes were used. The residual solvents were separated using 30 m × 0.25 mm × 0.25 μm DB1agilent 122-1032 capillary column and nitrogen as a carrier gas.

Solution preparation: Dimethyl sulfoxide was used as a diluent, it dissolves wide variety of substances and due to high boiling point it does not interfere with volatile solvents during HS-GC analysis. Stock solutions of methanol, acetonitrile, dichloromethane, benzene and toluene were prepared in 250 mL volumetric flask having 6000, 820, 1200, 4 and 1780 ppm concentrations, respectively. The concentration of stock solution is twice the ICH specification limit for given residual solvents. From the stock solution the standard solutions of residual solvents were prepared by diluting 25 mL stock solution to 50 mL. Standard of mixture is prepared by taking 1 mL standard solution of each residual solvent in 20 mL headspace vial. This mixture was used for the method development. Furthermore, samples having the concentration from 50 to 150 % of standard (allowed residual solvent limit) were prepared from standard stock solutions in 10 mL volumetric flask by appropriate dilution for determination of linearity and accuracy. For the analysis of residual solvents in EMPG, the samples were prepared by adding 1.0 g EMPG and 5.0 mL DMSO in 20 mL vial. The vial is sealed instantly with Teflon cap.

RESULTS AND DISCUSSION

Method Development: Since ethyl-3-methyl-3-phenylglycidate (EMPG) is used for human intake, the residual solvents in it should be quantified as per ICH guideline. This proposed method is assessed for separation of residual solvent from EMPG with quantification. The obtained results are compared with the corresponding specified limits of ICH standard guidelines.

Selection of solvent: DMSO and N,N-dimethyl acetamide are mostly preferable solvent for head-space due to their polar aprotic nature with high boiling points, good thermal stability and strong solubility for wide range of organic compounds. The N,N-dimethylacetamide has fishy odour and possess more health hazards like carcinogenicity as compared to DMSO. Furthermore, solubility of EMPG in DMSO is greater than 50 % (v/v). Thus DMSO is selected as a diluent in the HS-GC method development.

Optimization of chromatographic conditions: In order to separate and quantify the residual solvents, like methanol, acetonitrile, dichloromethane, toluene and benzene having intermediate polarity to non-polar nature, reverse phase capillary columns are considered. Two capillary column *viz*. HP-5 (5 % phenyl, 95 % methylpolysiloxane) and DB-1 (100 % dimethylpolysiloxane), both having same dimensions (30 m length × 0.32 mm ID, 1 μm film thickness), are used for method development. Although dichloromethane, toluene and benzene are separated on HP-5 column, the resolution of MeOH and CH_3CN could not be achieved. All of these residual solvents are separated on the DB-1 capillary column. Static HS sampling is typically used for the determination of residual solvents. This is done by heating samples to 60 °C for a definite time and then analyzing a mixture of vapors in headspace vial by GC equipped with the flame ionization detector (FID). Flame ionization detector is sensitive for carbon containing compounds and hence it is a suitable choice for analyzing the residual solvents. Hence HS-GC-FID method is used for quantification of residual solvents from EMPG. The headspace injector and GC conditions are provided in Table-1.

Different chromatographic parameters were optimized to obtain peak shape and resolution of the peaks within acceptable system suitability. In headspace high temperature is necessary to reach equilibrium concentration of residual solvents in gas phase and solution phase without degradation of residual solvents. Hence headspace oven temperature is optimized by considering lower boiling point (39.6 ºC) of dichloromethane as well as higher boiling point (110.6 ºC) of toluene. The equilibrium concentration of all residual solvents in gas phase with liquid phase is achieved at 60 ºC. To equilibrate the concentration of residual solvents in liquid and gases phases the sample is kept in oven for definite time. To achieve better accuracy in short span, different equilibrium time *e.g.*, 5, 10, 15 and 20 min are

TABLE-1
OPTIMIZED CHROMATOGRAPHIC CONDITION

GC conditions	
Total run time	20.5 min
Oven equilibration time	0.2 min
Oven temperature, flow rate and temperature gradient	45 °C for 8 min $\xrightarrow[15\ °C/min]{1.0\ mL/min}$ 120 °C $\xrightarrow[20\ °C/min]{1.0\ mL/min}$ 275°C
Split ratio	1:10
Injector temperature	200 °C
Detector temperature	250 °C
Total flow	14 mL/min
Head-space conditions	
Oven equilibration temperature	60 °C
Equilibration time	10 min
Transfer line temperature	90
Loop volume and temperature	1 mL and 70 °C
Vial volume	20 mL
Injection time	0.02 min
Injection volume	1000 µL
Vial standby flow (N_2)	20 mL/min

considered. The area of all five residual solvent was 46.58, 53.65, 53.70, and 53.69 mV, respectively. This indicates that the equilibrium is reached at 10 min and this equilibrium time is considered in further studies.

Headspace injection time plays vital role in resolution, response and sensitivity of detector, the chromatograms of EMPG and the residual solvents at different injection times are shown in Fig. 1. For 0.10 min injection time, all the peaks turn out to be quite broad. Moreover the first two peaks respectively of MeOH and acetonitrile are mixed. When injection time is 0.02 min, the resultant chromatogram shows good peak shape as well as all peaks residual solvents of are well resolved.

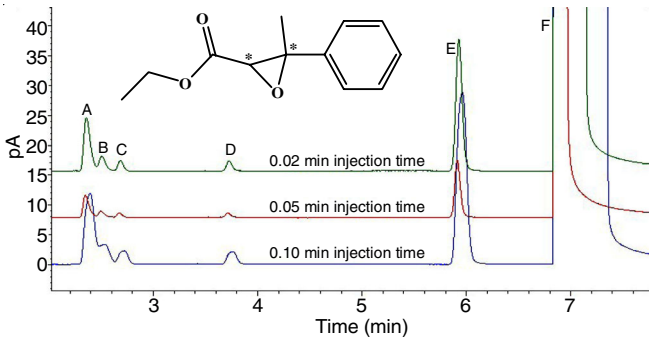

Fig. 1. Peak shape at different injection time (0.02 min: green, 0.05 min: red and 0.10 min: purple)

The high vapour pressure of aroma chemicals enabled their analysis using the head-space gas chromatographic methods [16]. Such head-space methods are routinely used for the odour analysis in the perfume and aroma industries [17,18]. Thus HS-GC-FID method residual solvent analysis has been extended to the estimation of four stereoisomers of EMPG. The separation of four EMPG isomers can be easily seen from the peaks at 16.97, 17.15, 17.43 and 17.58 min in Fig. 2.

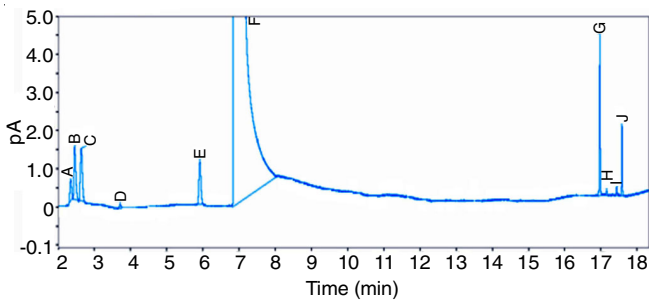

Fig. 2. Residual solvent standard mixture with EMPG standard sample, Peak resolution of methanol (A), acetonitrile (B), dichloromethane (C), benzene (D), toluene (E), diluents DMSO (F) and G-J enantiomers of EMPG

Method validation: The present HS-GC-FID method is validated as per ICH guideline for specificity, linearity, system precision, robustness and accuracy [19].

Specificity: Specificity is the power of method to resolve the analyte peaks. The specificity of this method determined by analyzing methanol, acetonitrile, dichloromethane, benzene, toluene, and DMSO individually as well as by mixing solvents under same experimental condition. The specificity parameters are shown in Table-2. The diluents do not show interference at the retention time of any residual solvents. The observed resolution of closest eluting MeOH and acetonitrile peaks is 1.84. All four enantiomers of EMPG are also well separated. The relative population of these isomers can estimated from the peak areas of these peaks, which turn out to be 1.00: 0.03: 0.06:0.41. Hence method was found to be specific.

Linearity and range: Linearity determines whether the test results obtained are directly proportional to concentration of analyte in the sample. The linear relationship evaluated across range of 50 to 150 % of ICH specified limit of residual solvents. The linearity data are shown in Table-3. The graphs of concentration *versus* peak area (figures are not shown) are linear and the regression coefficients 'R' for residual solvents are more than 0.99.

TABLE-2
RESOLUTION AND SYMMETRY DATA FOR RESIDUAL SOLVENT ANALYSIS IN EMPG

Retention time (min)	Peak name	Peak identity	Area (mV)	Height	Peak resolution	Peak theoretical plates	Peak symmetry	Peak tail factor
2.36	Methanol	A	3.54	3.24	–	10544	0.68	1.59
2.48	Acetonitrile	B	2.53	0.82	1.84	21814	0.64	1.23
2.66	Dichloromethane	C	4.07	1.33	2.45	18783	0.75	1.34
3.70	Benzene	D	1.31	0.20	13.79	40755	1.01	1.20
5.97	Toluene	E	33.20	8.83	25.92	60058	0.80	1.17
6.95	DMSO	F	728.08	97.15	8.419	33497	4.21	0.88
16.97	Z(2R,3R)-EMPG	G	6.00	5.57	120.56	5802574	0.95	1.02
17.15	Z(2S,3S)-EMPG	H	0.20	0.17	7.19	8944743	1.02	1.23
17.43	E(2S,3R)-EMPG	I	0.37	0.26	9.94	4690682	1.24	1.39
17.58	E(2R,3S)-EMPG	J	2.47	2.16	5.05	6228966	0.94	1.06

TABLE-3
LINEARITY OF RESIDUAL SOLVENTS IN THE RANGE FROM 50 TO 150 %

Range (%)	Area (mV)				
	Methanol	Acetonitrile	DCM	Benzene	Toluene
50	31.17	9.17	25.97	5.87	76.51
60	34.59	11.92	30.36	7.05	91.19
70	44.98	13.33	33.58	7.98	110.64
80	58.38	15.27	36.74	8.87	126.71
90	67.63	16.80	41.98	10.17	138.62
100	75.00	19.10	45.45	10.88	150.88
110	85.36	20.36	50.27	12.63	172.18
120	92.56	22.55	54.16	13.93	189.59
130	103.17	25.72	59.65	15.02	205.05
140	110.89	28.00	65.42	15.99	222.46
150	123.89	30.09	71.13	16.94	236.51
R^2	0.9960	0.9929	0.9928	0.9962	0.9982

Precision: It was determined by six replicate injections of standard samples. The % RSD and standard deviation were calculated for all individual solvent and mentioned in Table-4. The maximum precession was observed for aromatic solvent, where % RSD values are 0.16 and 0.19 for benzene and toluene, respectively. The low boiling solvents *i.e.*, CH_2Cl_2 and MeOH has larger (1.16 and 1.28) % RSD values. These values are well within the prescribed limits; hence method is precise for determination of residual solvent in EMPG.

TABLE-4
SYSTEM PRECISION DATA OF RESIDUAL SOLVENT ANALYSIS IN EMPG

Replication	Area (mV)				
	Methanol	Acetonitrile	DCM	Benzene	Toluene
1	73.99	18.08	44.06	10.79	150.17
2	74.16	18.11	45.08	10.81	150.93
3	75.14	18.25	45.66	10.81	150.85
4	75.83	18.35	45.47	10.80	150.88
5	75.73	18.17	45.39	10.82	150.69
6	74.01	18.25	45.45	10.77	150.83
Average	74.81	18.20	45.18	10.80	150.73
SD	0.86	0.10	0.58	0.02	0.28
RSD (%)	1.16	0.55	1.28	0.16	0.19

Accuracy: Accuracy, closeness of measured values and its actual or standard value, is determined by injecting known amount of residual solvent at three placebo levels *i.e*. 50, 100 and 150 % of standard solution. The recovery data of all five residual solvents are listed in Table-5. The recovery of MeOH, acetonitrile, CH_2Cl_2, benzene and toluene is observed to be 94.86-109.54 %, 100.71-108.47 %, 104.02-107.56 %, 99.40-107.57 % and 100.01-104.49 %, respectively. This confirms that the method is accurate for determination of residual solvent from EMPG in routine analysis.

Limit of detection and quantitation: Limit of detection (LOD) and limit of quantification (LOQ) have been determined on the basis of signal to noise ratio (S/N) [20]. LOD and LOQ mainly give the performance of instrument for given method. S/N ratio for LOD is greater than 3 and LOQ is greater than or equal to 10. LOD and LOQ measured by successive dilution of residual solvents for establish the minimum concentration at which it can be consistently detected and quantified. The results of residual solvent analysis are given in Table-6. The LOQ values are well below the ICH specification limit of the residual solvents.

TABLE-6
LOD AND LOQ (ppm) FOR RESIDUAL SOLVENTS IN EMPG

Residual solvents	Concentration (ppm)		
	LOD	LOQ	ICH limit
Methanol	0.036	0.184 ± 0.001	3000
Acetonitrile	0.015	0.098 ± 0.002	410
Dichloromethane	0.088	0.255 ± 0.002	600
Benzene	0.011	0.087 ± 0.005	2
Toluene	0.022	0.127 ± 0.005	890

Robustness: The term robustness referred as an ability of an analytical method to remain unaffected by small variations in method parameters (*viz*. mobile phase composition, column age, column temperature, column pressure) as well as influential environmental factors and characterize its reliability during normal usage. The robustness of proposed method determined by changing method parameter like flow rate and column oven temperature. The flow rate is changed by ± 5 % from original method, while the oven temperature is altered by ± 5 ºC. The data for these studies are listed in Table-7. The % RSD for each solvent less than 2.0, which affirms that the proposed method is robust.

Conclusion

The headspace GC method is developed for the quantification of residual solvents, *viz*. MeOH, acetonitrile, CH_2Cl_2, benzene and toluene in EMPG. The reverse pahse DB-1 capillary

TABLE-5
RECOVERY DATA OF RESIDUAL SOLVENT ANALYSIS IN EMPG

Concentration in % wrt limit	Replication	Recovery (%)				
		Methanol	Acetonitrile	Dichloromethane	Benzene	Toluene
50	1	93.33	100.71	108.75	108.66	101.53
	2	95.77	101.81	107.32	104.82	101.00
	3	95.48	99.61	106.60	109.23	100.87
	Average	94.86	100.71	107.56	107.57	101.13
100	1	100.25	99.41	100.59	100.73	100.10
	2	99.85	99.41	100.57	96.76	99.96
	3	99.98	101.61	100.30	100.72	99.98
	Average	100.03	100.14	100.49	99.40	100.01
150	1	109.78	109.20	103.63	104.54	104.61
	2	109.43	108.34	103.48	102.05	104.39
	3	109.41	107.88	104.95	100.08	104.47
	Average	109.54	108.47	104.02	102.22	104.49

TABLE-7
ROBUSTNESS PARAMETERS *i.e.* RETENTION TIME (RT, min) AND PEAK AREA (mV) OF RESIDUAL SOLVENTS

RS		Methanol		Acetonitrile		Dichloromethane		Benzene		Toluene	
		RT	Area	RT	Area	RT	Area	RT	Area	RT	Area
Set-I (original)	Average	2.36	74.81	2.54	18.20	2.75	45.18	4.01	10.80	6.78	150.73
	SD	0.00	0.86	0.00	0.10	0.00	0.58	0.00	0.02	0.00	0.28
	RSD (%)	0.03	1.16	0.02	0.55	0.00	1.28	0.01	0.16	0.01	0.19
Set-II (-5 %)	Average	2.44	74.15	2.59	18.31	2.80	45.31	3.98	9.25	6.52	150.51
	SD	0.00	0.46	0.00	0.32	0.00	0.27	0.00	0.08	0.00	0.23
	RSD (%)	0.08	0.62	0.02	1.73	0.07	0.61	0.01	0.85	0.07	0.16
Set-III (+5 %)	Average	2.24	74.60	2.37	18.62	2.53	45.38	3.45	8.52	6.38	150.50
	SD	0.00	0.17	0.00	0.28	0.00	0.62	0.00	0.05	0.00	0.30
	RSD (%)	0.05	0.23	0.02	1.51	0.10	1.36	0.04	0.59	0.04	0.20

column (100 % dimethyl polysiloxane), nitrogen carrier gas and FID detector are used. Along with the separation of residual solvents, the present HS-GC-FID method has resolved all the four enantiomers of EMPG. This method is successfully validated as per the criteria of ICH guideline for residual solvent analysis. Overall the method is rapid, accurate, linear, specific, robust and precise for residual solvent analysis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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