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Development and Validation of 2D GC-FID Method for Quantitative Analysis of *cis*- and *trans*-Hexyl Cinnamic Aldehyde and its Major Impurity 2-Hexyl-2-decenal

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Hexyl cinnamic aldehyde (HCA) is an aroma chemical used as a flavour and fragrance ingredient, which is synthesized in huge quantity worldwide. The hexyl cinnamic aldehyde present in the *trans*- and *cis*-forms with nearly 95:5 ratio. The 2-hexyl-dec-2-enal (HDEA) is process impurity in hexyl cinnamic aldehyde, which may amount upto ~ 2.0 %. However use of 2-hexyl-dec-2-enal is prohibited in the fragrance, while in flavours the Flavour and Extract Manufacturer Association (FEMA) has permitted its concentration only up to 5.0 ppm. Thus a simple and rapid two dimensional gas chromatographic method (2D-GC) for quantitative analysis of (*cis*-/*trans*-)hexyl cinnamic aldehyde and 2-hexyl-dec-2-enal have been developed. The non-polar and polar capillary columns are connected in series and the flame ionization detector with nitrogen carrier is used. The method yields a well separated *cis*-hexyl cinnamic aldehyde, *trans*-hexyl cinnamic aldehyde and 2-hexyl-dec-2-enal peaks from the other impurities. The method is validated according to the ICH guideline for precision, recovery, linearity, and limit of detection.

Keywords: Two dimensional gas chromatography, Hexyl cinnamic aldehyde, 2-Hexyl dec-2-enal.

INTRODUCTION

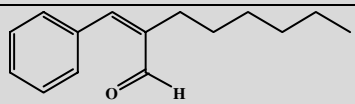
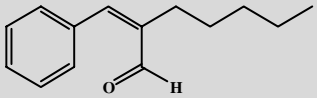
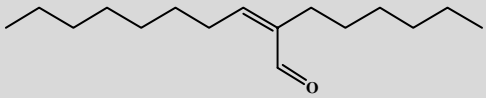
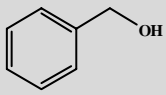
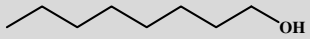
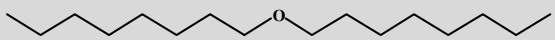
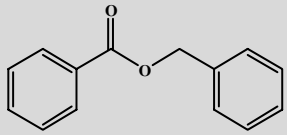
Hexyl cinnamic aldehyde (HCA), generically known as 2-hexyl-3-phenyl-2-propenal (Table-1) is an aroma chemical. The chamomile flower is one of the natural sources of HCA [1]. It is synthesized primarily from aldol condensation reaction between benzaldehyde and octanal [2]. This yields a HCA in the *trans*- and *cis*-forms with nearly 95:5 ratio. These isomers of HCA may differ in characteristic odor. The concentration of HCA in flavour and fragrance compound in different consumer products ranges from 0.7 to 17.1 %. It is unrestrictedly used in non-skin contact products like candles, air fresheners and room sprays [3]. It is also used in chemical, pharmaceutical and petroleum industries as precursor. The structure of HCA and the process impurities present in it are shown in Table-1. The 2-hexyl-dec-2-enal (HDEA) is a major impurity amounting upto ~ 2.0% (w/w). However, its use is prohibited in the fragrance, while as per flavour and extract manufacturer association (FEMA) amount of HDEA allowed in flavour is upto 5.0 ppm [4]. The presence of HDEA in higher concentration may adversely influence the quality of HCA [5], which makes it essential to control and analyze the HDEA precisely. The other impurities like 2-pentyl-3-phenyl-2-propenal, benzyl alcohol, and octanol may also present in HCA (Table-1). The separation of the *cis*-HCA, *trans*-HCA and HDEA from other impurities namely 2-pentyl-3-phenyl-2-propenal, benzyl alcohol, octanol and benzyl benzoate have not been reported yet. These impurities excluding HDEA and *cis*-HCA as well as *trans*-HCA can be separated on non-polar stationary phase; however resolution of the HDEA is achieved only on polar columns [6].

Thus in this paper, the accurate, fast, precise and reliable method is developed for separation of HDEA and *cis*- as well as *trans*-isomers of HCA in a single run by employing two dimensional gas chromatography (2D-GC) [7], where the non-polar and polar columns are used in series. The method is further validated according to ICH guidelines. The validation helps to builds a degree of confidence, not only for developer but also for users [8].

EXPERIMENTAL

Standard sample and test sample of hexyl cinnamic aldehyde (HCA) were received from Eternis Fine chemicals Limited, Kurkumbh, Pune. The analytical grade acetone as well as methanol and HPLC grade isopropyl alcohol were purchased from Merck, whereas octanaol, 2-pentyl-3-phenyl-2-propenal, 2-hexyl-dec-2-enal (HDEA), benzyl alcohol, *etc.* were purchased from sigma Aldrich. The Shimadzu Analytical balance, Agilent 7890B gas chromatography with flame

TABLE-1
CHEMICAL NAME AND STRUCTURE OF HEXYL CINNAMIC ALDEHYDE AND RELATED IMPURITIES

Label (chemical name)	Chemical structure	Category
2-Hexyl-3-phenyl-2-propenal (HCA)		F&F ingredient
2-Pentyl-3-phenyl-2-propenal		Impurity
2-Hexyl-dec-2-enal (HDEA)		Impurity
Benzyl alcohol		Impurity
Octanol		Impurity
di-Octyl ether		Impurity
Benzyl benzoate		Impurity

ionization detector and auto liquid sampler were used. All the instruments were calibrated during method development and validation. The isopropyl alcohol is used as solvent for sample preparation. The Agilent make columns having non-polar (HP-1) and polar (DB-wax) stationary phases with 30 m length, 0.25 µm internal diameter and 0.25 µm thickness were used [9].

RESULTS AND DISCUSSION

Method development: The gas chromatography is prime analytical technique for separation of volatile organic compound [10]. Its high efficiency allows the separation of component from the mixture in reasonable time. The evolution of stationary phase, capillary column in terms of length, thickness, as well as internal diameter and selection of carrier gas accelerated the development of the newer analytical methods. In order to achieve the separation of the relatively lipophilic 2-hexyl-dec-2-enal (HDEA) impurity from the hexyl cinnamic aldehyde (HCA), the polar columns like DB-wax and inert cap-FFAP are used. Both these column shows similar performance. The details of this Method-B are given in the Table-2 and the resultant chromatogram is displayed in the Fig. 1. The well separated HDEA (4.26 min) and HCA (7.07 min) peaks are observed, however the *cis*- and *trans*-isomers of HCA elute together. On the other hand when the non-polar column (HP-1) is used (Method A) these isomers were resolved, however the HDEA is eluted along with the *trans*-HCA at 8.49 min (Fig. 2) and for method parameters (Table-2). The variations in the different method parameters like flow rate, temperature, gradient were not able to resolve the HDEA from *trans*-HCA. The similar results were observed for the other non-polar RTX-5 and HP-5 column.

Thus in order to achieve the separation of HDEA as well as *cis*- and *trans*-HCA, the non-polar and polar columns were connected in series thereby leading to the 2D-GC separation. The resulting chromatogram is shown in the Fig. 3, where the

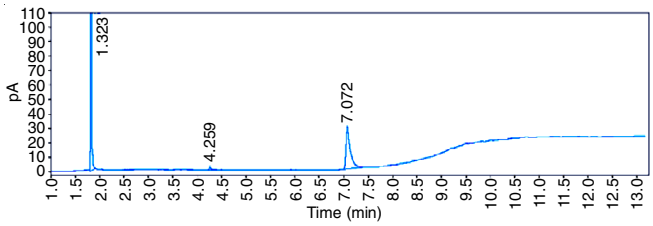


Fig. 1. Chromatogram of GC-FID Method-B with polar column showing HDEA (at 4.259 min) is separated from HCA (7.072 min)

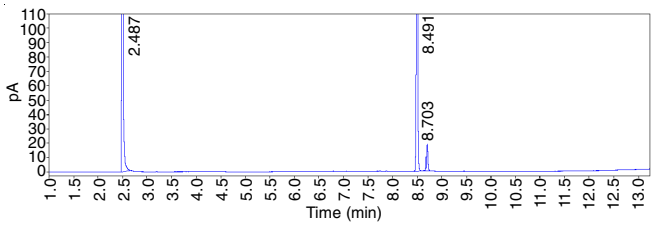


Fig. 2. Chromatogram of GC-FID Method-A with non-polar column showing separation of *trans*-HCA (8.49 min) and *cis*-HCA (8.70 min)

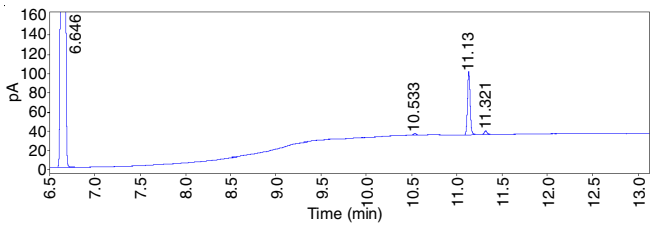


Fig. 3. Chromatogram 2D-GC FID method showing the separation of HDEA (10.53 min) from *trans*-HCA (11.13 min) and *cis*-HCA (11.32 min)

peak of HDEA (10.53 min), *trans*-HCA (11.13 min) and *cis*-HCA (11.32 min) are well resolved The corresponding resolution factors for these peaks are 10.80 and 3.50, respectively.

Method validation: The present 2D-GC method is validated as per ICH guideline for specificity, selectivity, precision, linearity, accuracy and robustness [11].

TABLE-2
CHROMATOGRAPHIC CONDITIONS FOR 2D GC

	Method A	Method B	2D-GC method
Instrument	Agilent 7890B with ALS	Agilent 7890B with ALS	Agilent 7890B with ALS
Column	HP-1 (30 M length × 0.25 µm ID × 0.25 µm thickness)	DB-wax (30 M length × 0.25 µm ID × 0.25 µm thickness)	HP-1 and DB-wax (both 30 M length × 0.25 µm ID × 0.25 µm thickness)
Detector	FID	FID	FID
Flow rate	Constant flow rate: 1.0 mL/min	Constant flow rate- 2.5 mL/min	Constant flow rate- 2.5 mL/min
Split ratio	30:1	30:1	30:1
Injector and detector temperature	280	280	280
Injection volume	0.1 µL	0.1 µL	0.1 µL
Column oven temperature	Initial temperature 100 °C, hold time 2 min, gradient of 30 °C/min till 210 °C, hold time 5 min, gradient of 20 °C/min till 260 °C, hold time 0.5 min	Initial temperature 160 °C, gradient of 30 °C/min till 210 °C, hold time 5 min, gradient of 20 °C/min till 260 °C, hold time 4 min	Initial temperature 160 °C, gradient of 30 °C/min till 210 °C, hold time 5 min, gradient of 20 °C/min till 260 °C, hold time 4 min
Run time	13.66 min	13.16 min	13.16 min
Detector air flow	300 mL/min	300 mL/min	300 mL/min
Detector hydrogen flow	30 mL/min	30 mL/min	30 mL/min
Carrier	Nitrogen	Nitrogen	Nitrogen

Selectivity: The selectivity refers to the extent to which the method can be used to determine particular analyte in mixtures or matrices without interferences from other components of similar behaviour. It simply means that at a given retention time only one component of the sample is being eluted. The selectivity of the method was evaluated by spiking plausible impurities under same experimental condition and distinguishing their retention time from the desired components in test sample [12].

Thus, the elution order of other impurities like octanol, benzyl alcohol, dioctyl ether, 2-pentyl-3-phenyl-2-propenal, benzyl benzoate, HDEA and *trans*-HCA as well as *cis*-HCA was studied by spiking these impurities one by one in the isopropyl alcohol and recording their chromatogram. The chromatogram of the spiked impurities in the test solution has been shown in the Fig. 4 and the retention times as well as the other peak factors are given in Table-3. The elution order is as follows: octanol, benzyl alcohol, dioctyl ether, 2-pentyl-3-phenyl-2-propenal, HDEA, *trans*-HCA, *cis*-HCA and benzyl benzoate. The resolution factor between *trans*-HCA and *cis*-HCA is 3.50 indicates the good separation of these peaks. The resolution factor for 2-pentyl-3-phenyl-2-propenal and HDEA is 4.49, rest all the peaks have even larger values of resolution factor. Therefore present 2D-GC method selectively resolve the targeted analytes *viz.* HDEA, *trans*-HCA and *cis*-HCA.

Linearity: Linearity is a capability of method to produce the response towards the analyte concentration in samples.

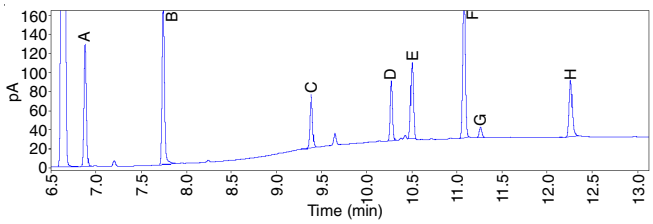


Fig. 4. Spiked chromatogram of all probable impurities for resolution. A: octanol, B: Benzyl alcohol, C: dioctyl ether, D: 2-pentyl-3-phenyl-2-propenal , E: HDEA, F: trans-HCA, G: cis-HCA, H: Benzyl benzoate

The method is linear if the response is directly proportional to the concentration of the analyte in the matrix within the range of analyte concentration of interest. The linearity of method is estimated for both HCA and HDEA by recording the chromatograms of their five standards of different concentrations in the desired range. The linearity plots of HDEA, *trans*-HCA and *cis*-HCA are shown in Fig. 5. The HDEA standards were prepared in concentration range from10 ppm to 50 ppm. The linearity for the detector response towards the concentration of HDEA yield a correlation coefficient of 0.997. Then the 4000 ppm solution of the HCA is prepared and the amount of HDEA present in it is detected using the aforementioned calibration curve. The concentration of the HDEA calculated from peak area (1.95) is turned out to be 44.4 ppm. Thus the amount of HDEA in the original HCA is turned out to be 1.11 %, in other words the purity of total HCA is 98.89 %. Furthermore from

TABLE-3
RESOLUTION OF OTHER IMPURITIES FROM HDEA, *trans*-HCA AND *cis*-HCA

Retention time (min)	Peak	Peak name	Peak symmetry	Peak resolution	Area	Peak selectivity	Peak theoretical plates
6.87	A	Octanol	0.86	4.77	231.98	1.04	335643
7.74	B	Benzyl alcohol	0.78	18.24	322.60	1.14	422281
9.38	C	di-Octyl ether	0.92	31.79	119.25	1.24	456672
10.27	D	2-Pentyl-3-phenyl-2-propenal	1.00	17.38	111.57	1.10	778580
10.50	E	HDEA	1.15	4.49	162.08	1.02	600172
11.07	F	*trans*-HCA	1.05	10.80	324.45	1.06	717335
11.26	G	*cis*-HCA	0.87	3.50	22.01	1.01	689343
12.26	H	Benzyl benzoate	0.88	10.62	136.59	1.09	696344

HCA = hexyl cinnamic aldehyde; HEDA = 2-hexyl-dec-2-enal

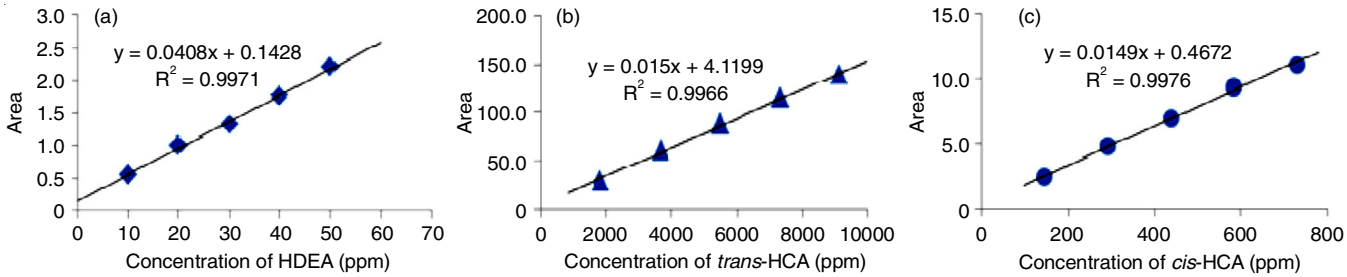


Fig. 5. Linearity graph for HDEA (a), *trans*-HCA (b) and *cis*-HCA (c)

the peak areas of the *trans*-HCA (60.60) and *cis*-HCA (4.86) their isomer ratio was calculated to be 92.57: 7.43. The total amount of HDEA, *trans*-HCA and *cis*-HCA present in 100 g of HCA stock is turned out to be 1.11 g, 91.56 g and 7.34 g, respectively. Using 99.89 % purity of HCA, the standard solutions of HCA are prepared to generation the calibration curve of *trans*-HCA and *cis*-HCA. Their respective concentration ranges are 1832-9160 ppm and 146-730 ppm. The correlation coefficient for linear regression is greater than 0.99 in both these cases. Therefore it is confirmed that the method is liner in the given range.

Precision: Precision provides an indication of agreement among individual test results when an analytical method is used repeatedly to multiple samples. Precision studies are performed when the entire analytical method procedure is finalized. System precision studies carried out on six replicate analysis of standard 10000 ppm HCA sample.

The method precision has been performed by replicate injecting test a sample (of 3000 ppm) for six times and the intermediate precision is determined by analyzing test sample at different day using same method and column. The data related to the precision study is represented in Table-4. The % RSD of six replicate HCA standards sample is 1.79, 1.58 and 1.42 for HDEA, *trans*-HCA and cis HCA respectively. The % RSD of method precision was observed 1.34, 1.21, and 1.48, respectively and the corresponding values for intermediate precision were 1.20, 1.32 and 0.70. The % RSD of all samples are less than 2.0, hence the method and system said to be precise.

Accuracy: The accuracy study indicates the closeness of the experimental values to the true value. Method accuracy is analyzed by carrying out recovery study of the sample which is known concentration of *trans*-HCA, *cis*-HCA and HDEA. The recovery data is represented in Table-4. Results of recovery study of test sample were compared with standard and it is determined using following formula:

$$\text{Accuracy} = \frac{\text{Area of sample} \times \text{Conc. of stanard}}{\text{Area of standard} \times \text{Conc. of sample}} \times 100$$

% RSD for the recovery of the *trans*-HCA, *cis*-HCA and HDEA is turned out to be 1.21, 1.48 and 1.34, respectively. The results obtained are within the limit (2.0 %) which indicates that the method is accurate.

Limit of detection and limit of quantification: There are several approaches to determine detection and quantification limit *i.e.* visual evaluation, signal to noise ratio (S/N) and statistical method based on calibration curve [13]. Here S/N was used to evaluate LOD and LOQ, this was done by serial dilution of HDEA and HCA in isopropyl alcohol till the consistent detectable lower concentration is achieved. The signal of samples with a known amount of low level analyte is compared with the noise of blank sample. From the accepted S/N for LOD are 3 and for LOQ is ≥ 10. The LOD and LOQ values for HDEA are 5.0 and 8.0 ppm, respectively and for the HCA are 10.0 and 15.0 ppm, respectively.

Robustness: The robustness of analytical method is determined by changing method parameter such as flow rate and column oven temperature. The flow rate was changed ± 0.5 mL/min and the column oven temperature is changed by ± 5 °C from 160 °C in original method. The data of the robustness studies are represented in Table-5. When flow rate and temperature is decreased, the retention times were prolonged by 0.75, 1.18 and 2.21 min, respectively for HDEA, *trans*-HCA and *cis*-HCA. On the contrary these retention times for increased flow rate and temperature were shortened by 2.36, 2.37 and 2.43 min, respectively. The % RSD for robustness studies were observed to be 0.99, 1.25 and 1.24, respectively for HDEA, *trans*-HCA and *cis*-HCA, when flow rate and temperature are decreased, whereas for increased flow rate and temperature corresponding % RSD values are 1.85, 1.62 and 1.74. There was insignificant change in values of the system suitability

TABLE 4
COMPARATIVE DATA OF PRECISION AND RECOVERY STUDIES OF *trans*-HCA, *cis*-HCA AND HDEA

Replicate	Retention time (min)			Area									Recovery standard (%)		
				Standard sample			Test sample			Test sample (day 2)					
	trans-HCA	*cis*-HCA	HDEA	*trans*-HCA	*cis*-HCA	HDEA	*trans*-HCA	*cis*-HCA	HDEA	*trans*-HCA	*cis*-HCA	HDEA	*trans*-HCA	*cis*-HCA	HDEA
1	11.16	11.35	10.55	138.37	11.12	5.03	40.35	3.23	1.56	40.99	3.99	1.96	97.20	96.82	103.38
2	11.16	11.35	10.55	142.80	11.37	5.05	40.07	3.35	1.55	41.33	3.99	1.95	96.53	100.42	102.72
3	11.16	11.35	10.55	143.78	11.49	5.14	40.39	3.23	1.52	40.83	3.92	1.94	97.30	96.82	100.73
4	11.16	11.35	10.55	141.51	11.37	5.08	41.18	3.22	1.55	41.81	3.98	1.91	99.20	96.52	102.72
5	11.16	11.35	10.55	140.48	11.02	4.91	41.2	3.27	1.57	41.93	3.95	1.96	99.25	98.02	104.04
6	11.16	11.35	10.55	143.10	11.36	4.92	41.07	3.25	1.52	42.24	3.98	1.97	98.94	97.42	100.73
%RSD	0.01	0.01	0.00	1.42	1.58	1.79	1.21	1.48	1.34	1.32	0.70	1.20	1.21	1.48	1.34

HCA = hexyl cinnamic aldehyde; HEDA = 2-hexyl-dec-2-enal

TABLE-5
ROBUSTNESS STUDY DATA OF *trans*-HCA, *cis*-HCA AND HDEA

Replicate	Set-I: Set-I: Initial temperature 155 °C and flow 2.0						Set-II: Initial temperature 165 °C and flow 3.0					
	Retention time (mn)			Area			Retention time (mn)			Area		
	HDEA	*trans*-HCA	*cis*-HCA	HDEA	*trans*-HCA	*cis*-HCA	HDEA	*trans*-HCA	*cis*-HCA	HDEA	*trans*-HCA	*cis*-HCA
1	11.91	12.53	12.76	1.03	36.02	1.89	9.55	10.16	10.33	1.16	39.61	3.61
2	11.91	12.53	12.76	1.00	36.63	1.85	9.55	10.16	10.32	1.16	39.62	3.66
3	11.91	12.53	12.76	1.00	36.47	1.89	9.55	10.16	10.33	1.20	39.82	3.62
4	11.90	12.53	12.75	1.01	36.43	1.84	9.55	10.16	10.32	1.15	38.10	3.59
5	11.90	12.53	12.76	1.00	37.27	1.85	9.56	10.16	10.32	1.13	38.92	3.48
6	11.91	12.53	12.76	1.01	36.06	1.85	9.56	10.16	10.32	1.16	39.39	3.65
SD	0.004	0.001	0.004	0.01	0.46	0.02	0.002	0.000	0.001	0.02	0.64	0.06
%RSD	0.030	0.010	0.034	0.99	1.25	1.24	0.022	0.04	0.013	1.85	1.62	1.74

HEDA = 2-hexyl-dec-2-enal; HCA = hexyl cinnamic aldehyde

criteria like tailing factor, theoretical plates and % RSD. The values of these studies are well within acceptance limit.

Conclusion

Accurate, linear, specific, robust, and precise 2D GC method is developed and is successfully validated as per the criteria of ICH guideline for analytical method validation for the quantitative analysis of flavour and fragrance ingredient hexyl cinnamic aldehyde and its impurity 2-hexyl-dec-2-enal. The HDEA peak is well resolved from HCA and its other known impurities. The method is linear with correlation coefficient being greater than 0.99, and system, method and intermediate precision are evaluated and % RSD was found within limit. Robustness also does not show significant change for the system suitability. The present 2 dimensional gas chromatographic method is useful for routine quantitative analysis of both the *cis*- and *trans*- isomers of HCA as well as its major impurity HDEA.

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REFERENCES

1. T. Rustemeyer, P. Elsner, S.M. John and H.I Maibach, Kanerva's Occupational Dermatology, *Aromatherapist*, **118**, 1266 (2012).
2. (a) G. Shi-long, *China Surf. Det. Cosm. Syn.*, **1**, 17 (2006); (b) Z. Li-rong and Y. Feng-ping, *Chem. World*, **12**, 10 (2014).
3. International Fragrance Association (IFRA); α-Hexyl Cinnamic aldehyde, 47th amendment (2013).
4. S.M. Cohen, S. Fukushima, N.J. Gooderham, S.S. Hecht, L.J. Marnett, I.M.C.M. Rietjens, R.L. Smith, M. Bastaki, M.M. Mcgowen, C. Harman and S.V. Taylor, 27th publication by the Expert Panel of Flavor and Extract Manufacturers. Association (FEMA), GRAS 27, Food Technology (2011).
5. D. Rowe, Chemistry and Technology of Flavor and Fragrances, Wiley-Blackwell Publishing Ltd, UK, edn 1, p. 351 (2009).
6. Sigma-Aldhrich, GC Column Selection Guide, Achieve Optimal Method Performance, Nicholas, Walter, p. 22.
7. C. Vendeuvre, R. Ruiz-Guerrero, F. Bertoncini, L. Duval and D. Thiébaut, *Oil Gas Sci. Technol.*, **62**, 43 (2007); https://doi.org/10.2516/ogst:2007004.
8. V. Ravichandra, S. Shalini, K.M. Sundram and R. Harish, *Int. J. Pharm. Pharm. Sci.*, **2(Suppl 3)**, 18 (2010).
9. Column Selection Principles, Agilent J&W GC Column Selection Guide, pp. 19.
10. A.G. Piantanida and A. R. Barron, Principles of Gas Chromatography, OpenStax-CNX, (1.2) pp. 1-12 (2014).
11. ICH Expert, ICH Harmonized Tripartite Guideline, Validation of Analytical Test Procedures and Method, Q2(R1), pp. 17 (2005).
12. J. Vessman, R.I. Stefan, J.F. van Staden, K. Danzer, W. Lindner, D.T. Burns, A. Fajgelj and H. Müller, *Pure Appl. Chem.*, **73**, 1381 (2001); https://doi.org/10.1351/pac200173081381.
13. S.B. Puranik, V.R. Pawar, N. Lalitha, P.N.S. Pai and G.K. Rao, *Orient. J. Chem.*, **24**, 529 (2008).