



**Review Article**

**STEPS TO BE CONSIDERED DURING METHOD DEVELOPMENT AND VALIDATION FOR  
ANALYSIS OF RESIDUAL SOLVENTS BY GAS CHROMATOGRAPHY**

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**Abstract:** Residual solvents are potentially undesirable substances in all type of formulations when present above the permissible limits given by ICH guidelines in 1997 and their regulation must be properly in order to avoid their deleterious health effects. This review provides some important steps for method development and validation, to detect and quantitate residual solvents. Several steps which is to be considered for method development like column section (stationary phase and dimensions: column id, length, and film thickness), carrier gas selection (Nitrogen, Helium, flow rate), temperature programing (Initial temperature, initial hold, ramp rate, final temperature, and final hold) are discussed.

**Keywords:** Residual Solvents, Gas Chromatography, Method Development and Validation

**Introduction**

Diethylene glycol (bis [2-hydroxyethyl] ether), are chemically classified as glycols. Diethylene glycol is widely used in chemical industries as solvents or antifreeze products that may cause neuro- and nephrotoxicity in humans. Poisoning associated with Diethylene glycol ingestion through manufactured pharmaceutical products has been reported by the World Health Organization (WHO). Incidents in the United States, Nigeria, and Haiti were due to inadequate control of raw materials and final products.<sup>1</sup>

DEG is a derivative of ethylene glycol with both compounds causing acute renal failure. DEG, like ethylene glycol, can increase the osmolal gap. However, unlike ethylene glycol, DEG does not cause a metabolic acidosis. DEG has occurred as a contaminant or has been used a substitute for propylene glycol and glycerine. In 1937, 105 patients died in the United States when 72% DEG was used as a diluent in the antibiotic preparation called Elixir Sulphanilamide. It has been associated with several international epidemics having numerous fatalities over the past 60 years.<sup>2</sup> Residual solvents in pharmaceuticals defined as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products. The solvents are not completely removed by practical manufacturing techniques.

**Classification of Residual Solvents by Risk Assessment**

The term "Tolerable Daily Intake" (TDI) is used by the International Program on Chemical Safety (IPCS) to describe exposure limits of toxic chemicals and "Acceptable Daily Intake" (ADI) is used by the World Health Organization (WHO) and other national and international health authorities and institutes. The new term "Permitted Daily Exposure" (PDE) is defined in the present guideline as a pharmaceutically acceptable intake of residual solvents to

avoid confusion of differing values for ADI's of the same substance.<sup>3</sup>

**Limits of Residual Solvents**

As per ICH Guidelines, residual solvents have been classified into the four types (Table-1) and based on their toxicity criteria and also have given acceptable limits for them (Table-2).

Class 4 solvents including methyl isopropyl ketone, methyl tetrahydrofuran, petroleum ether, trichloroacetic acid, trifluoroacetic acid, isopropyl ether, 1, 1-diethoxypropane, 1, 1-dimethoxymethane, 2, 2-dimethoxypropane and isooctane were not found to have any toxicity data, so no limits were imposed on them.<sup>4</sup>

**Analytical Techniques for Residual Solvent Determination**

Different analytical techniques are available for the estimation of residual solvents in herbals including gravimetric analysis i.e. Loss on Drying (LOD), Thermo Gravimetric Analysis (TGA), Differential Thermal Analysis (DTA) or Differential Scanning Calorimetry (DSC), Thermal Desorption (TD), GC-MS, Chemsensor and some spectrometric and spectroscopic procedures. But gas chromatography based test procedures are the most popular and are chemically specific for residual solvents.<sup>5</sup>

**METHOD SCREENING**

**Column Selection**

A column is of course, the starting and central piece of a chromatograph. An appropriately selected column can produce a good chromatographic separation which provides an accurate and reliable analysis. An improperly used column can often generate confusing, inadequate, and poor separations which can lead to results that are invalid or complex to interpret. There are over 10,000 compounds that can be analysed by GC and over 400 GC capillary columns.

It is a challenge for a column manufacturer to give detailed applications.  
column selection guidelines to meet such a wide variety of

**Table 1: Classification of residual solvents**

Residual Solvent Class	Indication	Claim	Remark
Class 1	Solvents to be avoided	Known human carcinogens strongly suspected human Carcinogens environmental hazards	If unavoidable, then their levels should be restricted as per the limits given by ICH Guidelines
Class 2	Solvents to be limited	Non-genotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratogenicity	Permitted daily exposures (PDE) are given to the nearest 0.1 mg/day.
Class 3	Solvents with Low toxic potential	No health based exposure limit is needed	Less toxic in acute or short term studies; 50 mg/day (5000 ppm) or less was found acceptable.
Class 4	Solvents with No toxicological Data	No health based exposure limit is needed	Do not have any toxicological data and hence no prescribed limits are there.

**Table 2: Limits of Residual Solvents and Toxicity Criteria as provided by ICH [ICH Q3C]**

Class 1		Class 2		Class 3	
Solvent	Limit (ppm)	Solvent	Limit (ppm)	Solvent	Limit (%w/w)
Benzene	2	Acetonitrile	410	Acetic acid	0.5
Carbon tetrachloride	4	Chlorobenzene	360	Acetone	0.5
1,2-Dichloroethane	5	Chloroform	60	Anisole	0.5
1,1-Dichloroethane	8	Cyclohexane	3880	1-Butanol	0.5
1,1,1-Trichloroethane	1500	1,2-Dichloroethene	1870	2-Butanol	0.5
		Dichloromethane	600	Butyl acetate	0.5
		1,2-Dimethoxyethane	100	t-Butylmethyl ether	0.5
		N,N-Dimethylacetamide	1090	Cumene	0.5
		N,N-Dimethylformamide	880	Dimethylsulfoxide	0.5
		1,4-Dioxane	380	Ethyl acetate	0.5
		2-Ethoxyethanol	160	Ethyl ether	0.5
		Ethylene glycol	620	Ethyl formate	0.5
		Formamide	220	Formic acid	0.5
		Hexane	290	Heptane	0.5
		Methanol	3000	Isobutyl acetate	0.5
		2-Methoxyethanol	50	Isopropyl acetate	0.5
		Methylbutyl ketone	50	Methyl acetate	0.5
		Methylcyclohexane	1180	3-Methyl-1-butanol	0.5
		N-Methylpyrrolidone	4840	Methylethyl ketone	0.5
		Nitromethane	50	Methylisobutyl ketone	0.5
		Pyridine	200	2-methyl-1-propanol	0.5
		Sulfolane	160	Pentane	0.5
		Tetralin	100	1-Pentanol	0.5
		Toluene	890	1-Propanol	0.5
		1,1,2-Trichloroethane	80	2-propanol	0.5
		Xylene	2170	Propyl acetate	0.5
				Tetrahydrofurane	0.5

**Column Characteristics**

A column is characterized by its stationary phase and dimensions (column internal diameter, length, and film thickness or more precisely phase ratio). All of these variables directly affect separation to different degrees in elution order, retention time, peak resolution, and peak shape/height. Additionally, column performance is largely characterized by column inertness, plate numbers and column bleed in a chromatographic application. Therefore, indirectly or directly impacting how accurate and reliable the analysis performs. Among these variables, stationary phase is the most influential and effective variable that leads to a good separation on a properly maintained column.

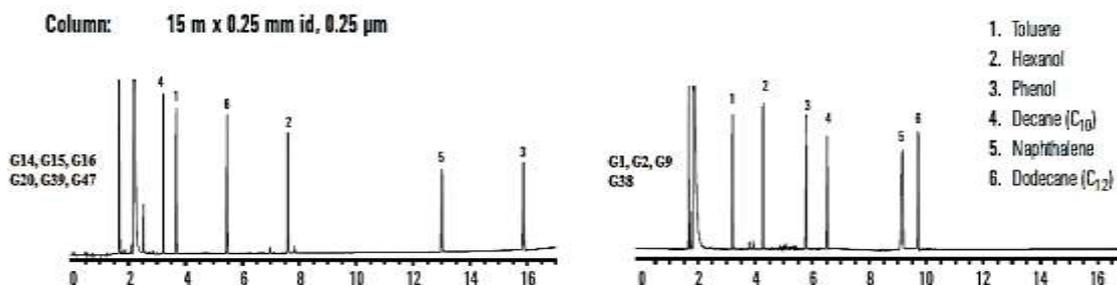
An optimized chromatographic separation begins with the column. The selection of the proper capillary column for any application should be based on four significant factors which are stationary phase, column internal diameter, film thickness, and column length. The differences in the chemical and physical properties of injected organic compounds and their interactions with stationary phase are the basis of separation process. When strength of the

analyte-phase interactions differs significantly for two compounds, one is retained longer than the other. How long they are retained in the column (retention time) is a measure of these analyte-phase interactions. Changing the chemical features of the stationary phase alters its physical properties. Two compounds that co-elute (do not separate) on a particular stationary phase might separate on another phase of different chemistry.

**Selection of Stationary Phase**

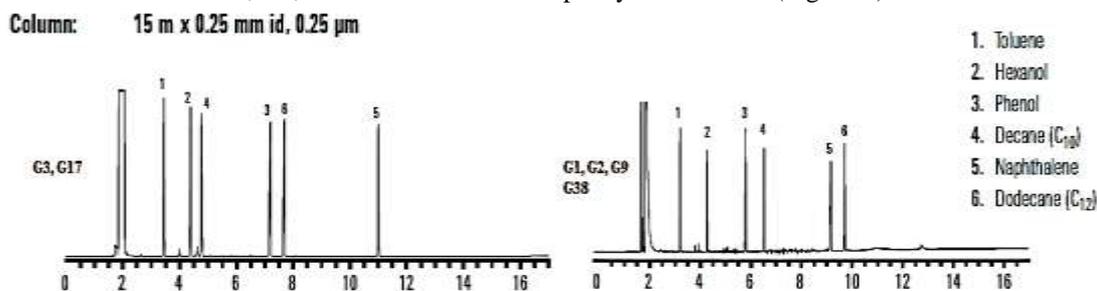
When selecting a column, first determine the samples characteristics to match the columns stationary phase. Stationary phases are in general divided into 3 categories first non-polar second mid-polar and third Polar. Stationary phases are further categorized by Siloxane (non-polar and mid-polar) and Polyethylene glycol (PEG or polar).

G1, G2 and G38 (100% Methyl Polysiloxane) does not undergo hydrogen bonding interactions. The change in the elution order of Hexanol and Phenol with G14, G15, G16, G20, G39 and G47 (Polyethylene glycol) is a combination of dipole and hydrogen bonding interaction (Figure-1).



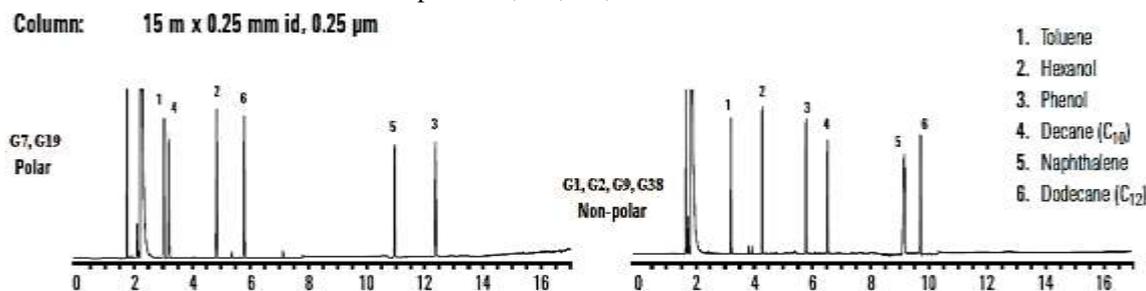
**Figure-1: Hydrogen Bonding Interactions**

The aromatics increase in retention relative to hydrocarbons for the G17 columns. G3 and G17 contain 50% phenyl substitution. G1, G2, G9 and G38 contain no phenyl substitution (Figure-2).



**Figure-2: Phenyl Content Retention**

The alcohols (polar) increase in retention relative to hydrocarbons (non-polar) for the G7 and G19 column phase and is more polar G1, G2, G9, and G38.



**Fig: Polarity–Retention Relationship**

### Selection of Column Internal Diameter (i.d.)

**High efficiency:** Column id plays two contradicting roles in separation. The efficiency of a capillary column, measured in plates (n) or plates per meter (n/m), increases as the i.d. of the column decreases. This is one of the basic principles behind fast GC but decreased sample loading capacities. When a column is overloaded with sample, the plate number is decreased greatly. If the sample to be analysed contains many analytes, or has analytes that elute closely together, the most narrow i.d. capillary column that is practical should be selected. Note that very narrow bore columns, such as 0.10 or 0.18 mm i.d., may require specialized equipment, such as GC with a pressure regulator that allows higher column head pressure.<sup>6,7</sup>

### Finding the Best Carrier Gas Average Linear Velocity

Determining the best average linear velocity is fairly easy and only involves a small amount of trial and error. Hydrogen provides the best resolution in shortest amount of time. Helium provides similar resolution, but at a longer analysis time. Nitrogen is not recommended for use with capillary columns due to the extremely long analysis times.

When using helium as the carrier gas, try an initial average linear velocity of 30 cm/sec. If better resolution is desired, reduce the velocity to not less than 25 cm/sec; however, the analysis time will be increased. If a shorter analysis time is desired, increase the velocity to 35-40 cm/sec; be aware of potential resolution losses at these higher linear velocities. Average linear velocities of 30-35 cm/sec are used for many analyses when using helium as a carrier gas.

When using hydrogen as the carrier gas, try an initial average linear velocity of 60 cm/sec. If better resolution is desired, reduce the velocity to not less than 50 cm/sec; however, the analysis time will be increased. If a shorter analysis time is desired, increase the velocity to 70-80 cm/sec; be aware of potential resolution losses at these higher linear velocities. Average linear velocities of 60-70 cm/sec are used for many analyses when using hydrogen as carrier gas.

Upon comparing the chromatograms at various average linear velocities, retention and resolution differences will be noticeable. Sometimes different average linear velocities are best for different peaks within the same chromatogram. In these cases, a compromise velocity is usual selected. Except with nitrogen, small changes in the average linear velocity (<2 cm/sec) rarely result in significant changes in resolution. When experimenting with average linear velocities, try values that are different by at least 3-4 cm/sec.<sup>8,9</sup>

### Oven Programming

Isothermal temperature condition involves maintaining a constant oven temperature throughout the GC run. Isothermal temperature conditions are used for solutes with similar retention. Retention differences for dissimilar solutes can be quite severe for isothermal temperature conditions. Peak width rapidly increases with retention for isothermal conditions. For these reasons, isothermal temperature conditions are only suitable for limited number of analyses. Most analyses require the use of a temperature program. A temperature program involves heating the oven at controlled rate during the run. This allows the faster analysis of solutes with dissimilar retention, and there is very little peak

broadening with an increase in retention. The primary disadvantages of temperature program are the more difficult development process and cool down time between analyses. There are no secrets or tricks to finding the best temperature program for an analysis. Usually some trial and error is involved.

If numerous attempts at different temperature programs have not resulted in satisfactory peak resolution, a different approach may be necessary. Some compounds cannot be separated with a particular stationary phase with any reasonable temperature program, thus a different stationary phase may be necessary. Sometimes improving efficiency may be the answer. Optimizing the carrier gas average linear velocity, improving injector efficiency, or using a more efficient column dimension may provide the desired resolution.

### Warning when Adjusting Temperature Program

When changing a temperature program, confirmation of peak identities in the new chromatogram is essential. Peak retention orders can shift upon a change in the temperature program (called peak inversions). Peak miss-identifications or an apparent loss of a peak (actually co-eluting with another peak) are common results of undetected peak inversions.

### Developing a Temperature Program

Using a linear temperature program as a starting point if previous analysis information is not available to use as a guide, the first program development step is to try a simple, linear temperature program. This provides information on the retention characteristics of the solutes. Start with an initial temperature of 50°C, a ramp rate of 10°C/min, a final temperature equal to the isothermal temperature limit of the column and a final hold time of approximately 30 minutes. The long final hold time is used to ensure all of the solutes elute from the column. The program can be stopped several minutes after last solute has eluted from the column. This may occur before the final temperature is reached. After obtaining a chromatogram using the simple, linear temperature program, the next steps are to adjust the various program components to obtain adequate resolution and the shortest analysis time.

### Adjusting the Initial Temperature and Hold Time

To improve the resolution of earlier eluting peaks, decrease the initial temperature or increase the initial hold time. Decreasing the initial temperature usually results in the largest resolution improvement, but analysis times are substantially increased. In addition, cool down times between runs can be significantly increased especially when cooling below 50°C. The resolutions of later eluting peaks are minimally affected by lowering the initial temperature especially for longer length columns. If excessive resolution is obtained with the original linear temperature program, increase the initial temperature to reduce resolution and analysis time. The resolution of later eluting peaks may also be reduced upon increasing the initial temperature.

Increasing the initial hold time often improves resolution of earlier eluting peaks; however, improvement is smaller than those obtained with lowering the initial temperature. The resolution of later eluting peaks is minimally affected with a

change in the initial hold time. Lowering the initial temperature and increasing the initial hold time can be combined to improve resolution of earlier eluting peaks. Hold times should be limited to 5 minutes or less if possible. Peaks eluting during the later portion of hold time may start to broaden, thus making resolution more difficult to achieve.

#### Adjusting the Ramp Rate

The resolution of peaks eluting in the middle of the chromatogram can be altered by change in ramp rate. If there is excessive peak resolution, the ramp rate can be increased to reduce resolution and the analysis time. If there is insufficient resolution, decrease the ramp rate, but there will be an increase in the analysis time. Better resolution of later eluting peaks often occurs when decreasing the ramp rate. Only change in ramp rate by about 5°/min each time. Much larger or smaller alterations usually cause massive or insignificant changes, respectively. Changes in initial temperatures and times can be combined with ramp rate changes to affect a large section of the chromatogram. Multiple ramp rates can be used to affect smaller regions of the chromatogram. For example, if 5°/min was good for earlier portion of the chromatogram and 15°/min was better for a later portion, both ramp rates can be used within a single program.

Another option to alter resolution of peaks in the middle of a chromatogram is to use a mid-ramp hold. A mid ramp hold is a several minute isothermal portion somewhere during a temperature ramp. For example, the temperature program of 50-100°c at 10°c/min, 100°c for 3 min, 100-300°c at 10°c/min contains a mid-ramp hold. To determine a suitable hold temperature, calculate the oven temperature range when the first peak of interest is eluting. Hold times of 2-5 minutes are most effective. Shorter or longer times often have no, or a detrimental effect on peak resolution. Try several different temperatures and hold times since small changes in the times and temperatures can be significant. Use a mid-ramp hold only if other temperature program changes were not effective.

#### Final Temperature and Time

Stop the temperature program shortly after last peak has eluted from the column. If column's isothermal temperature limit is reached and peaks are still eluting, a final hold time is necessary. Only use a final hold time if the temperature limit is reached. Any peaks that elute during isothermal temperature conditions substantially increase in width as peak retention increases.

Extracted samples often contain compounds that elute after the last solute of interest. The final temperature and/or hold time need to be large enough to ensure elution of these compounds. Higher final temperatures or longer hold times should be tried until it is certain that all solutes elute from column for every run. Column contamination will occur if portions of previously injected samples remain in the column during later injections.<sup>10</sup>

#### METHOD VALIDATION

The following are typical analytical performance characteristics which may be tested during methods validation

**Accuracy:** Accuracy is the measure of exactness of an analytical method, or closeness of agreement between the value which is accepted either as a conventional, true value or an accepted reference value and the value found.<sup>11</sup>

**Precision:** The precision of an analytical method is the degree of agreement among individual test results obtained when method is applied to multiple sampling of a homogenous sample. Precision is a measure of reproducibility of whole analytical method (including sampling, sample preparation and analysis) under normal operating circumstances. Precision is determined by using method to assay a sample for sufficient number of times to obtain statistically valid results (i.e. between 6 - 10). The precision is then expressed as the relative standard deviation.<sup>12</sup>

$$\%RSD = \frac{\text{Std. Dev} \times 100\%}{\text{Mean}}$$

**Linearity:** Linearity is the ability of analytical procedure to obtain a response that is directly proportional to concentration (amount) of analyte in sample. If method is linear, the test results are directly or by well-defined mathematical transformation proportional to concentration of analyte in samples within a given range. Linearity is usually expressed as the confidence limit around the slope of the regression line.<sup>13</sup>

**Specificity:** Specificity of an analytical method was determined by injecting blank solution of pure under the same experimental conditions. No peak was observed from the chromatogram obtained by injecting as a blank.<sup>14</sup>

**Lower Limit of Detection and Lower Limit of Quantification:** The lower limit of detection (LLOD) is defined as the lowest concentration of an analyte in a sample that can be detected, not quantified. It is expressed as a concentration at a specified signal to noise ratio, usually 3:1. The lower limit of quantitation (LLOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of method. The ICH has recommended a signal to noise ratio 10:1. LLOD and LLOQ may also be calculated based on standard deviation of the response (SD) and slope of calibration curve(S) at levels approximating the LOD according to the formulae: LLOD=3(SD/S) and LLOQ=10(SD/S).<sup>15</sup>

**Selectivity:** Selectivity is the ability of an analytical method to differentiate and quantify the analyte in presence of other components in sample. For selectivity, analyses of blank samples should be tested for interference, and selectivity should be ensured at the lower limit of quantification (LLOQ).<sup>16</sup>

**Ruggedness:** Different analyst with different instruments and column defines ruggedness.<sup>17</sup> Ruggedness is normally evaluated during method development, typically by the originating laboratory, before collaborating with other laboratories and is a measure how well a method stands up to less than perfect implementation. In any method there will be certain stages, which, if not carried out sufficiently

carefully, will have a severe effect on method performance, and may even result in the method not working at all. These stages should be identified, usually as part of method development, and if possible, their influence on method performance evaluated using 'ruggedness tests', sometimes also called 'robustness tests'. This involves making deliberate variations to the method, and investigating the subsequent effect on performance. It is then possible to identify the variables in the method, which have the most significant effect and ensure that, when using the method, they are closely controlled. Where there is a need to improve the method further, improvements can probably be made by concentrating on those parts of the method known to be critical. Ruggedness tests are normally applied to investigate the effect on either precision or accuracy.<sup>18</sup>

**System Suitability:** System performance parameters of developed GC method should be determined by injecting system suitability solution in six replicates. Parameters such as number of theoretical plates, tailing factor, resolution and relative standard deviation should be determined.<sup>19</sup>

## REFERENCES

1. Patricia Baffi, Suria Elneser, Myrna Baffi & Matilde De Melin. Quantitative Determination of Diethylene Glycol Contamination in Pharmaceutical Products. *Journal of AOAC International*, **2000**; 83 (4): 793-801.
2. Robert H. Williams, Steve M. Shah, Jack A. Maggiore & Timothy B. Erickson. Simultaneous Detection and Quantitation of Diethylene Glycol, Ethylene Glycol, and the Toxic Alcohols in Serum using Capillary Column Gas Chromatography. *Journal of Analytical Toxicology*, **2000**; 24: 621-624.
3. ICH harmonised tripartite guideline on impurities: guideline for residual solvents (ICH Q3C (R5)), International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 4 February **2011**.
4. Munish Garg *et al.* Regulatory and Analytical Aspects of Residual Solvents in Ayurvedic Formulations-A Recent Update. *International Journal of Research and Reviews in Pharmacy and Applied Science*, **2012**; 2(5):938-949.
5. Witschi C, Doelker E. Residual solvents in pharmaceutical products: acceptable limits, influences on physicochemical properties, analytical methods and documented values. *Eur J Pharm Biopharm*, **1997**; 43(3):215-242.
6. Agilent J & W GC Column Selection Guide. Available at: [http://www.google.co.in/url?sa=t&rct=j&q=column+selection+guide+gc&source=web&cd=5&cad=rja&ved=0CEgQFjAE&url=http%3A%2F%2Fwww.crawfordscientific.com%2Fdownloads%2Fpdf\\_new%2FGC%2FAgilent\\_J%26W\\_GC\\_Column\\_Selection\\_Guide.pdf&ei=J6phUff-GIbprQfS84CgDw&usg=AFQjCNGBnCO2jbZ\\_ZAhRx\\_LEU8PD2OLhvw](http://www.google.co.in/url?sa=t&rct=j&q=column+selection+guide+gc&source=web&cd=5&cad=rja&ved=0CEgQFjAE&url=http%3A%2F%2Fwww.crawfordscientific.com%2Fdownloads%2Fpdf_new%2FGC%2FAgilent_J%26W_GC_Column_Selection_Guide.pdf&ei=J6phUff-GIbprQfS84CgDw&usg=AFQjCNGBnCO2jbZ_ZAhRx_LEU8PD2OLhvw)
7. GS-Tech Column Selection Guideline. Available at: [http://www.google.co.in/url?sa=t&rct=j&q=Conventional+Wisdom+for+Column+Selection&source=web&cd=1&cad=rja&ved=0CDIQFjAA&url=http%3A%2F%2Fwww.labsphere.biz%2Fpdf%2Fconsumables%2Fgs-tek%2FGS-tek-column-selection.pdf&ei=XLNhUej6IcLTrQeruIDIBQ&usg=AFQjCNH7HszqX\\_zmDg-wDV8HJbUulQjd4A](http://www.google.co.in/url?sa=t&rct=j&q=Conventional+Wisdom+for+Column+Selection&source=web&cd=1&cad=rja&ved=0CDIQFjAA&url=http%3A%2F%2Fwww.labsphere.biz%2Fpdf%2Fconsumables%2Fgs-tek%2FGS-tek-column-selection.pdf&ei=XLNhUej6IcLTrQeruIDIBQ&usg=AFQjCNH7HszqX_zmDg-wDV8HJbUulQjd4A)
8. L.S. Ettre and J.V. Hinshaw. Selecting Carrier Gases and Conditions. *J. High Resolut, Chromatogr*, **1997**; 20(9): 471-481.
9. J. Hinshaw. Selecting Carrier Gases and Conditions. *Lc•Gc Int.* **1995**; 8(11): 631-635.
10. Agilent Technologies, Inc. August 7, 2007; Printed in USA. Available at: [http://www.google.co.in/url?sa=t&rct=j&q=gc+method+development+agilent&source=web&cd=3&cad=rja&ved=0CEEQFjAC&url=http%3A%2F%2Fwww.chem.agilent.com%2FLibrary%2FSupport%2FDocuments%2F3964554214.pdf&ei=prJhUb3zEMulrQfuzYHQBQ&usg=AFQjCNHgT0xD\\_jZp\\_RJf9SCAU15oK49G4g](http://www.google.co.in/url?sa=t&rct=j&q=gc+method+development+agilent&source=web&cd=3&cad=rja&ved=0CEEQFjAC&url=http%3A%2F%2Fwww.chem.agilent.com%2FLibrary%2FSupport%2FDocuments%2F3964554214.pdf&ei=prJhUb3zEMulrQfuzYHQBQ&usg=AFQjCNHgT0xD_jZp_RJf9SCAU15oK49G4g)
11. Narasimharao R., Laxmi Raj A., Sanjay Kumar Ch., Kapil Ch., Chaitanya M. Method Development and Validation of Duloxetine Hydrochloride By RPHPLC. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, **2011**; 2(2): 1335-1349.
12. Validation of Compendial Assays-Guidelines'pharmacopeial Convention, Rockville, MD, **1985**
13. Vibha Gupta *et al.* Development and Validation of HPLC Method - A Review. *International Research Journal of Pharmaceutical and Applied Sciences*, **2012**; 2(4): 17-25
14. V. Varun Raj, G. Pramod, N. Naresh Babu. Estimation of Residual Solvents in Clopidogrel Bisulphate by Using Chromatographic Techniques (GC). *International Journal of Research in Pharmaceutical and Biomedical Sciences*, **2010**; 1(2): 92-96.
15. Mohammad T. Zzaman, Sadaf J. Gilani, K. Nagarajan, Shadab A. Siddiqui. HPLC Method Development and Validation for Pharmaceutical Analysis-A Review. *Internationale Pharmaceutica Scientia*, **2012**; 2(3): 13-21.
16. Guidance for Industry, Bioanalytical Method Validation; U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center For Veterinary Medicine (CVM), May **2001**, BP.
17. P. Susantakumar, Ajay Gaur, Piush Sharma. Development and Validation of LC-MS/MS Method for Estimation of Acyclovir in Pharmaceutical Dosage Form. *Pharmacophore, An International Research Journal*, **2011**; 2(4): 210-224.
18. Kapil Kalra (2011). Method development and validation of analytical procedures, quality control of herbal medicines and related areas, prof. Yukihiro shoyama (ed.), ISBN: 978-953-307-682-9, Intech, available from: <http://www.intechopen.com/books/quality-control->

of-herbal-medicines-and-related-  
areas/methoddevelopment-and-validation-of-  
analytical-procedures

19. Rajan V. Rele, R. N. Mali. Determination of Residual Solvents in Citalopram Hydrobromide by Gas Chromatography. *American Journal of Pharmtech Research*, **2012**; 2(4):618-625.