Gas Chromatography optimization using experimental design and surface response methodology

By

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Abbreviation

CCD--- Central Composite Design

COC--- Cool-on-column

DoE--- Design of Experiment

ECL--- Equivalent Chain Length

ECD--- Electron Capture detector

FAMEs--- Fatty Acid Methyl Esters

FPD--- Flame Photometric detector

GC---Gas Chromatography

H---Plate height

ISO--- isothermal model detector

MS--- Mass Spectrometer detector

N ---Number of theoretical plate

NPD--- Nitrogen Phosphorous detector

PD--- Pressure drop

PPC--- Peaks per Carbon

PTGC---Programmed temperature Gas Chromatography

RMSE---Root Mean Square Error

SN --- Separation Number

TCD---Thermal Conductivity Detector

VD--- Van Deemter equation / model

VD+Int --- Expanded van Deemter model (with interaction)

Abstract

Gas Chromatography (GC) is an analytical method for the separation of enormous range of routine applications, to analyse very complex mixtures of Petroleum, such us different proteins in the human body, and natural products. To achieve good enough separation with reasonable analysis time, optimisation is the best approaching techniques in chromatography. The work is focused to : investigate and optimized extra-column effects, thereafter investigate suitable range of temperature rates in temperature-programmed in 10-60m column length. For isothermal GC, investigate how well different dispersion models fit experimental data, and how the equations change with column length. Pilot study for both oven temperature with condition of 10, 30, 60m were conducted. Main experiment, for PTGC, 25 levels of carrier gases (H_2, He, $N_{2)}$ velocities, 5 levels of temperature rates and 10-60m column lengths were the variables in the main study. The variables were set with frame reference of pilot study for both oven temperature condition. Same column dimension and 10 levels of carrier gas velocities were the important constraint for isothermal GC. For the optimization of chromatographic conditions, experimental design and response surface methodology, Chrombox C and D were employed. Split ratio (1:200), asymmetry ($\leq$ 1.25ng), starting temperature (PTGC, 60°C), oven temperature (isothermal, 210°C) sampling frequency (>10Hz) and make up gas flow rate (40mL/min) are the conditions which were investigated and set values for pilot and main studies.

In isothermal GC, five dispersion models for plate height were calculated, in six different capillary column lengths with different level of pressure drop. Depends on the value of Overall R^2 and RMSE, Golay model with interim pressure drop is good fit models of plate height; irrespective of column dimension and carrier gas. In PTGC, 10°C per void time is claimed to give optimal rates for a given capillary column. The relevance of the advice was compared to the data from this work by calculating PPC/t_{topt} for the five rates for each column (10-60m). From the relationship PPC/t_{topt} and ratelength, all the relationship is increasing in all cases and there is no maximum in PPC/t_{topt} is found within the conditions tested. Although the development in PPC/t_{topt} seems similar for all columns and carrier gases, the proposed optima are very different. In the same ratelength and for all carrier gases, the performance of column length in every 10m transition was evaluated for isothermal and PTGC. For PTGC, when we shift from 40 m to 50 m, 50 m to 60 m are the transition where maximum 0.4% and minimum 0.3% PPC/t_{topt} values are observed respectively. Switching off carrier gas, for all employed column lengths (10m-60m), helium by hydrogen, nitrogen by helium the efficiency will be increased by 7.3%, decreased by 16.2% respectively. On the contrary, the time required for the analysis, for the above switching off carrier gases, are decreased by 5.4% and increased by 9.8% while in isothermal the efficiency is increased not more than 4.13%. Carrier gas transition effect (analysis time) in isothermal is much more significance than programmed temperature but the efficiency is dominated in programmed temperature. Column cut off deviation is measured since we used by cutting off single 60m column length. Highest deviation (12%) is recorded in the last cut off 10m while the lowest (2%) is in the first cut off in every 10m nominal length (60-10m).

Key words : Chromatography, model, efficiency, ratelength, deviation, column.

1. Introduction

Chromatography is derived from the Greek words chromos (colour) and graphy (write) and was originally used in the theory of colours. This term had been employed by The Russian botanist and biochemist M. S. Tswett (1872–1919) [1], in the beginning of the twentieth century, to describe the separation of leaf pigments. According to the International Union of Pure and Applied Chemistry (IUPAC) [2],

Modern chromatography "It is a physical method of separation in which the components to be separated are distributed between two phases, stationary phase and mobile phase". Mobile phase may be a gas, liquid, or supercritical fluid and is moved by gravitation, capillary forces, or pressure to transport the analyte through the column, the heart of separation where separation is being carried out. The separation mechanism to separate the complex matrix, distribution of the sample between stationary and mobile phase, can be based on adsorption, affinities of analyte towards stationary phase, solubility, ion exchange, size exclusion, or selective interactions. This analytical method is typically used for identification purpose from retention data of the analyte and quantification purpose from the response, peak area, of the analyte [3]. Depending on the nature of stationary phase and the state of the mobile phase, chromatography can be categorised in different types. When the mobile phase is liquid, the chromatography is liquid chromatography and also when the mobile phase is gas, the name of the chromatography is called gas chromatography.

Gas-Solid Chromatography (GSC) and Gas-Liquid Chromatography (GLC) are the most common and applicable gas chromatography with solid and liquid stationary phases [4] respectively. In these two chromatographys, the separation column can be either packed, solid particle or liquid stationary phase coated onto a particle support, or open tubular; adsorption and solubility are the separation mechanism for GSC and GLC respectively.

In 1941, Martin and Synge were the two scientists who introduced Gas Liquid chromatography as analytical method for analysis of complex sample [5]. Its mobile phase is a gas. Since couples decade years back, capillary gas chromatography (GLC) has become very essentially tool in analytical chemistry for enormous range of routine applications, to analyse very complex mixtures of Petroleum, different proteins in the human body, natural products, volatile and semi-volatile.

The interest of reducing cost per analysis (i.e. higher laboratory throughput, better utilization of high-cost equipment) and the significance of improving efficiency have forced the separation scientist to give their time and effort to conduct research on the area of separation science [6].

For the area of capillary gas chromatography, there has been a demand for an increased speed of analysis, lots of approaches had been explored including reduction of column inner diameter persuasively demonstrated. In 1977, Gaspar [7] and his co-workers came up with a novel injection technique.

Band broadening of chromatogram is the other very important issue in capillary chromatography that has been given special interest. The effect is the outcome of the average sum of both on column and extra column (stationary phase, column dimension, column temperature, ramp rate, elution temperature & pneumatic parameters, injection mode, connection of column, injected sample amount, detection, data acquisition and other electronics) [8, 9].

The influence of temperature on the speed of analysis and resolution between two consecutive chromatogram peaks was presented by Harris and Habgoodr [10]. The effect of temperature on peak height (band broadening) was described by De Wet and Pretorius [4]. For higher temperature, the times required for analysis become lower; whereas the efficiency of the column becomes too poor. And to overcome the above two main concerns, separation scientist had been conducting plenty of researches and method optimization within the on-column and off-column parameters of gas Chromatography. The best performance of chromatography separation can be achieved by changing column dimension, mobile phase, column temperature, flow rate, pressure. Chromatography optimization was described using H and H/u and suggested by Purnell [11], in 1959. The lower, H and H/u, parameters, the better efficiency and fast analysis time respectively. Column optimization increased N to obtain minimum H; and to optimize speed of analysis obtaining shortest void time was the best condition. Ettre and Sandra [12] had examined chromatographic efficiency and speed of analysis with respect to varying column diameter and stationary film thickness. The influence of high inlet pressures, sample capacity and sensitivity of smaller column diameter on the analysis of time and efficiency had been reported. The impact of the outlined limitation did not bring significance problem on the efficiency and short analysis time; smaller column diameter, better efficiency and short analysis time too [13]. For the optimization criteria (H or H/u), column optimization is not the only issue, there are other optimization parameters (carrier gas velocity, pressure, temperature etc). In mid 1990s, Blumberg [14] proposed to use gas flow rate as optimized parameters to obtain H_{min} (in which flow rate, F_{opt} dependent on temperature and column diameter but independent column length. The effect of oven ramp rate in temperature programmed gas chromatography GC, on column efficiency, under the optimization constraints (pressure, phase ratio, constant flow and constant column length) was claimed by L. M. Blumberg, & M. S. Klee [15]. To have a reduced analysis time in temperature programmed GC, increased the heating rate; however, an increase in the heating rate cause to punish to record lower column efficiency, column peak capacity.

The existence of an optimal heating rate was suggested by Giddings [16] who proposed several simple semi empirical formula for evaluation of the optimal heating rate. Though it is practically unclear. Henri Snijders and his colleagues [17], 1995, have reported a numerical method to predict retention times and peak widths of chromatogram in capillary gas chromatography. This method is based on the

thermodynamic values (enthalpy and entropy terms) from Kovats retention indices for both in isothermal and programmed temperature capillary gas chromatography.

In the optimization of GC, installing short column cannot be grantee to obtain better efficiency and of course the time for analysis will be too short; do not forget as there are important constraints, instrumental contributions, the injection techniques, and electronic time/sampling frequency/ constants that should be taken in to account in GC [18]. These are some of the condition that will be addressed by this study.

1.1 Objective

This work is the direct continuation of Svein A. Mjøs & Habtewold D. Waktola (2015) Optimizing the relationship between chromatographic efficiency and retention times in temperature-programmed gas chromatography. The objectives of the work are:

1) Investigate how different instrument settings affect and extra-column affects the performance of GC system and find conditions to minimize these.
2) Investigate the range of suitable temperature rates in temperature-programmed GC for different carrier gases (H_2, He, N_2) and column lengths (10-60 m).
3) Investigate whether there exist an ideal temperature rate for a specific column length in temperature-programmed GC.
4) For isothermal GC, investigate how well different dispersion models fit experimental data, and how the equations change with column length and carrier gas properties.

2. Theory

2.1 Gas chromatography (GC)

GC is a very vital separation technique for both qualitative identification and quantification of materials that are able to volatile. The mobile phase of GC is gas (helium, hydrogen, nitrogen, Argon) and solid or liquid stationary phases which are either solid support or coated on the side of the capillary column. In Figure 1, the main skeletons of gas chromatography are itemized as follow: gas supply, gas flow control, sample injection system, oven, and detector [19].

Gas supply

The carrier gas or mobile phase in GC is an essential, but limiting, facet in separations. Carrier gas is the means to move constituents of a sample through the column and yet the choice of possible gas is mainly economically, its contribution to the efficiency and analysis time, as well the type of detectors going to be used. Unlike liquid chromatography (where a wide selection of mobile phase compositions may be possible), very little can be gained in separations through altering the mobile phase composition to influence the partition coefficient (k) or separation factor (α) in GC.

Pneumatic controls

Gas flow rate and gas supply through the separation column is determined by the head pressure. The pressure is monitored by Pneumatic device**.** Controlling is usually required to regulate the gas coming into the instrument and then to supply the various parts of the instrument. However, as the oven temperature increases (with temperature programming), the viscosity of the gas will increase and the flow rate will fall in nonlinear manner. Under such conditions, flow rates may slow at high temperature and both separation speed and efficiency may suffer. Pressure drop can influence on the operation condition of inlet split flow, inlet septum purge flow, detector air flow, detector hydrogen flow, detector make-up gas flow [19, 20].

Sample inlets

It is the component of the GC system where the samples (gaseous or liquid) are being injected using the device using a micro-litter syringe and auto-sampler. It is the region of analyte vaporization. Reasonable inlet types are chosen depending on the sample being analyzed, the separation column dimension being employed. Some of the inlet methods are: Split, splitless, and on column. Unsound injection system (split ratio, inlet temperature, the amount of samples) can bring a problem of band broadening and loss of resolution [21].

Column Oven:

Oven is the component of GC that is being used to control the temperature in the system. The oven heats rapidly to give excellent thermal control. And also the oven is cooled by fan and vent arrangement usually located at the rear side of the oven. Above all the most important feature of a modern oven are capable of controlling the oven temperature in different elements of the system and able to conduct the thermal energy between the separation column and the heating element. column is hanged inside the oven, cage, that is able to support the GC column and to prevent it touching the oven walls as this can damage the column prevents [19, 20].

Detector

The detector responds to a physicochemical property of the analyte, amplifies this response and generates an electronic signal for the data system to produce a chromatogram. There are number of detector types exist and the choice is based mainly on analyte chemistry, carrier gas type and sensitivity. The most common detectors in GC are: Flame Ionization (FID), Electron Capture (ECD), Flame Photometric (FPD), Nitrogen Phosphorous (NPD), Thermal Conductivity (TCD) and Mass Spectrometer (MS) [21].

Flame ionization detector

FID is also called carbon selective detector. It provides a near universal response to organic compounds with exception compounds containing a single carbon atom bonded to oxygen or sulphur, certain nitrogen oxides, inorganic gases. It is working with low detection limits, long-term stability, and simplicity of operation, low dead volume, a fast response and its response mechanism is mass dependant [19, 20, 22].

The detector response is resulted from the reaction of carbon-containing compounds (come out from column) with a hydrogen-air flame where the sample analyte is being converted to carbon dioxide, water, electron and ions, the ions will ultimately create an electric current and it is depending on the components present in the sample (reaction equation 1). The hydrogen-air flame alone creates few ions, but when an organic compound is burned there is an increase in ions produced. A polarizing voltage attracts these ions to a collector located near the flame. The current produced is proportional to the amount of sample being burned. This current is sensed by an electrometer, converted to digital form, and sent to an output device. The sampling rate of analogue to digital is the worthy condition on the band broadening of the chromatogram; faster speed allows minimum peak widths while slow rate causes to displayed broad peak width [19, 20].

C-**compounds (sample) + H_2 + air $\rightarrow$ CO_2 + H_2O + e+ ions** ---- reaction equation **1**

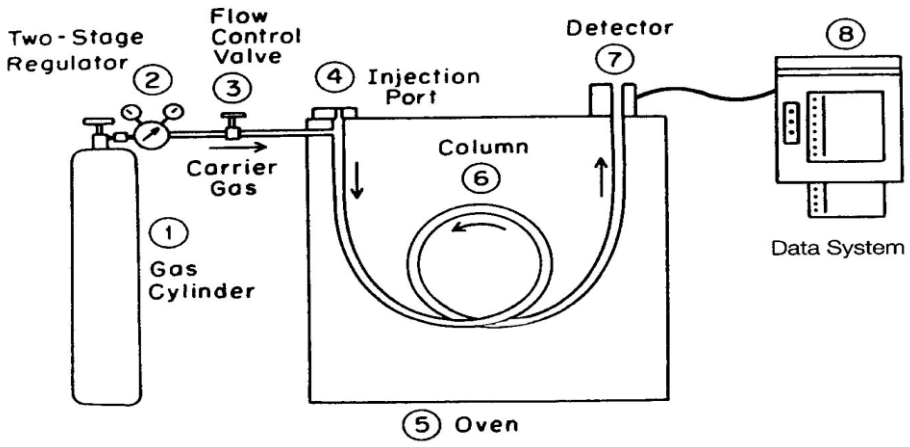

Figure 1: GC diagram **[19]**

2.1.1 Isothermal gas chromatography

Scott [23] studied the effect of temperature under isothermal conditions on the efficiency, resolution, and analysis time of capillary columns. This is the operation condition in which the column temperature is constantly maintained throughout the analysis. The major disadvantages of this operation set up are: A decreased sample throughput, long run times, broad peaks for late eluting components exposed to carryover effect from heavier components (Figure *7*: a) [20].

Sample is introduced to gas chromatography system via injection system and the sample moved to the column by carrier gas; separation is taken place in the column, heart of separation, and the data domain is being encoded by detector, in which gives the response of the components eluted from the column [24]. Separation of sample components into a series of chromatographic peaks, each representing a single component in the sample mixture, is the main goal of chromatography (Figure 2). The separation between two chromatographic peak$_1$ and peak$_2$ can be measured by resolution (R_s). It is quantitative measurement of the ability of the column to separate two analyte [21], which is expressed by Equation 1**.**

$$R_s = \frac{2(t_{R2} - t_{R1})}{(w_{b2} + w_{b1})} \quad (1)$$

Where t_{R1}, t_{R2} are the retention times of compound 1 and 2 respectively. Where w_{b1} and w_{b2} are the corresponding peak width at baseline.

For a successful separation there must be different retention of the analytes. Retention is measured by the retention factor, *k*, which is defined by Equation 2.

$$k = \frac{n_S}{n_M} \quad (2)$$

where n_S and n_M are the number of molecules of the analyte in the stationary phase and mobile phase, respectively.

The holdup time, dead time or void time (t_M) is the time the mobile phase uses through the column and is defined by Equation 3.

$$t_M = \frac{L}{u} \quad (3)$$

where *L* is the column length and *u* is the average mobile phase velocity.

In isothermal gas chromatography the retention factor of the analyte can be calculated from the retention time, t_R, and holdup time, t_M, Equation 4.

$$k = \frac{t_R - t_M}{t_M} \quad (4)$$

While t_M is the time the analyte spend in the mobile phase before it elutes, the time spent in the stationary phase is given by the numerator in Equation 4, which is referred to as the adjusted retention time, t'_R, Equation 5.

$$t'_R = t_R - t_M \quad (5)$$

The difference in retention between two compounds is referred to as chromatographic selectivity and measured by the separation factor, α, which is defined by Equation 6.

$$\alpha = \frac{k_2}{k_1} = \frac{t'_{R2}}{t'_{R1}} \quad (6)$$

Since α is always the retention factor of the last peak (highest *k*) divided by the retention factor of the first peak (lowest *k*), its value will never be lower than 1, and a value of 1 means zero chromatographic selectivity.

The separation of two peaks is not only dependent on the difference in retention, but the peak widths are also important. Peaks in chromatography are usually assumed to be normally distributed in shape, and their widths are defined by the standard deviation, σ, the peak width at baseline, w_b, defined as 4σ, or width at half peak height w_h, which is 2.355σ (Figure 2). Peak with relative to retention time is referred to as chromatographic efficiency and given by the plate number, *N*, Equation 7.

$$N = \left(\frac{t_R}{\sigma}\right)^2 = 16\left(\frac{t_R}{w_b}\right)^2 \quad (7)$$

The Purnell equation [25] summarizes the three factors that is necessary for chromatographic separation, retention, selectivity and efficiency, and is given by Equation 8.

$$R_s = \left(\frac{\sqrt{N_2}}{4}\right)\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{1 + k_2}\right) \quad (8)$$

It can be seen from the Purnell equation that there will be no resolution between the peaks is *N* is zero, α is one or *k* is zero. It can be seen from Figure 3 how *N*, α and k is

affecting the different factors in the equation. The last two factors both have a limit of 1, and approximately half the potential is achieved already with α equal to 2 and k equal to 1, which means that these values should not be too low, but there is little to gain in resolution by having very large values. The factor with *N* has no upper limit, but the gain in resolution is only proportional to the square root of *N*.

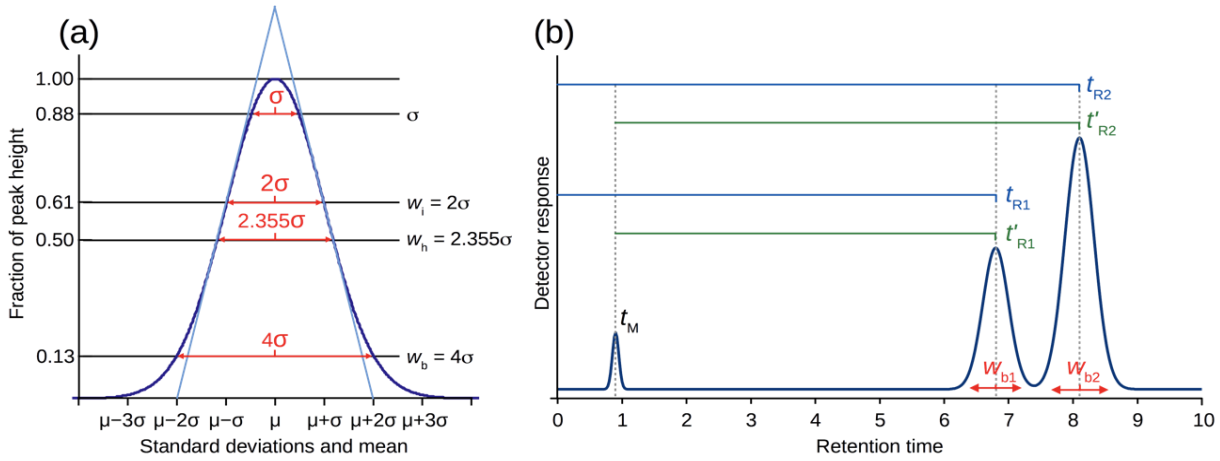

Figure 2:Chromatogram (left) and anatomy of a peak (right)

The retention time is directly proportional to the column length and inversely proportional to the average linear velocity of the mobile phase according to this equation.

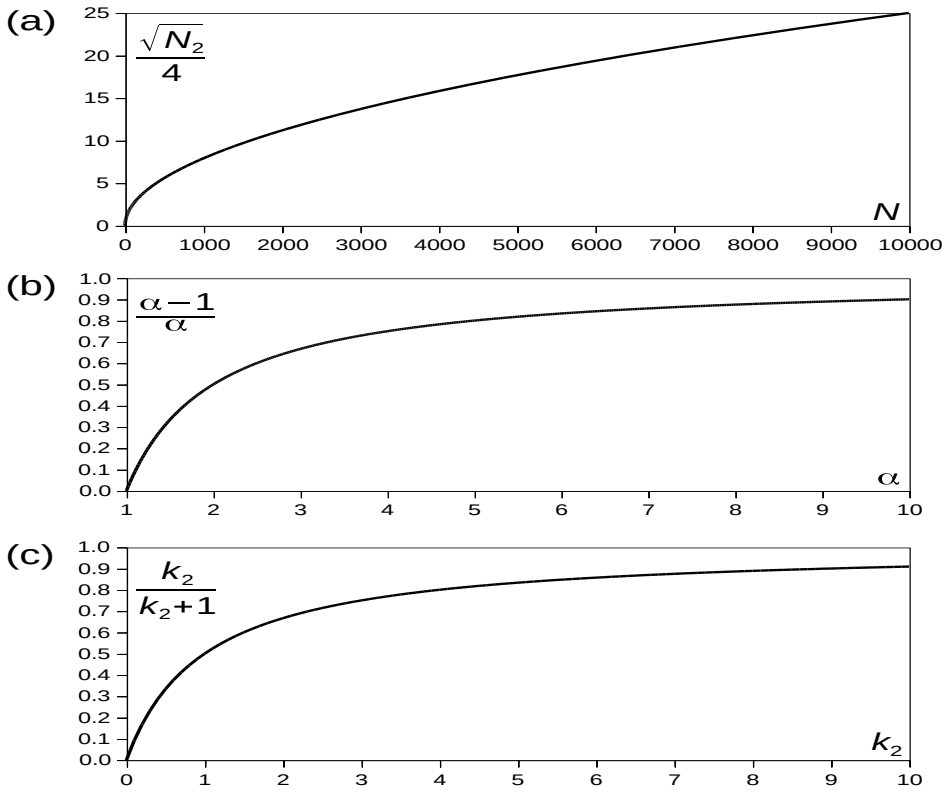

Figure 3: factors of Purnell equation (a) N, number of theoretical plates (b) α, relative retention (c) k, capacity factor (k) and (c) relative retention

2.1.1.1 The distribution constant and factors affecting the retention

The retention factor is influenced by the nature of stationary phase, column temperature, and dimension of column. The retention factor is dimensionless and expresses how long a solute is retained in the stationary phase compared to the time needed to transport the carrier gas through the column

While the retention factor (Equation 2) is the fraction of the number of molecules in the stationary and mobile phase (or the fraction of the masses since the molar mass of the analyte is the same in the two phases), the distribution constant, K_c, is the fraction of the concentrations, Equation 9.

$$K_c = \frac{c_S}{c_M} \tag{9}$$

The phase ratio is the fraction of the volumes of the two phases, and is for a capillary column given by Equation 10.

$$\beta = \frac{V_M}{V_S} \approx \frac{0.25\, d_c}{d_f} \tag{10}$$

where V refers to volumes, d_c is the inner diameter of the capillary column, and d_f is the thickness of the stationary phase.

The relationship between k, K_c, and β is given by Equation 11.

$$k = \frac{K_c}{\beta} \tag{11}$$

It follows from the equations above that k is proportional to K_c and the film thickness, d_f, and inversely proportional to the column diameter d_c. The equations also tell that for a column with a given stationary phase and certain dimensions, the retention factors can only be manipulated by adjusting the distribution constant. The distribution constant depends on thermodynamic parameters, as shown in Equation 12.

$$\ln K_c = \frac{-\Delta G^0}{RT} \tag{12}$$

Where R is the ideal gas constant, ΔG^0 is the change in Gibbs free energy for the evaporation of the compound from the stationary phase, and T is the Kelvin temperature. The numerator in this equation (Gibb's free energy) depends on the chemical interactions between the analytes and the stationary phase, which cannot be manipulated if the phase and the analytes are given. From the above equations it can therefore be seen that the only way to adjust K_c and k in a system is by adjusting the temperature.

2.1.1.2 Factors affecting selectivity and separation factor (α)

Selectivity is the measure of how good a column can separate two substances by their different chemical properties for a given mobile phase (carrier gas). It is usually measured by the separation factor, α (Equation 6). Retention of analyte molecules occurs due to interactions with the stationary phase. Therefore, interactions between the stationary phase and analyte are of great importance. The most important interactions between analytes and stationary phases are including:

- Dispersive force.

  They are common forces to all molecules whether or not they possess a permanent dipole moment; typical for non-polar solutes, e.g. aliphatic and aromatic hydrocarbons. Dispersion forces increase with the molecular mass of the molecules, which results in a higher boiling point.

- Dipole Induction and dipole–dipole

  Forces are directed forces between polar molecules (molecules with dipole, with a permanent dipole) and polarizable molecules.

- Hydrogen bonding

  The hydrogen bond is the strongest electrostatic dipole–dipole interaction and resulted from the attractive force between a hydrogen atom covalently bonded to a very electronegative atom such as N, O, or F atom and another very electronegative atom [26].

Stationary phase polarity is determined by the polarity of the substituted groups and their relative amounts. It has a pronounced effect on compound retention and separation. For compounds of similar volatility, greater retention is obtained for solutes with polarities similar to the stationary phase. In other words, polar compounds are more strongly retained by apolar stationary phase than a less polar stationary phase,and vice versa.

Temperature is the major control variable used in gas chromatography. Elevated temperature decreases retention for all compounds, but it also can have minor effects on selectivity. Peak positions do not always maintain their relative position as the temperature is increased [24, 27] .

2.1.1.3 Factors affecting efficiency

The number of theoretical plates (*N*) is a measure of column efficiency in isothermal GC. A theoretical plate is defined as the average distance travelled in one distribution step, or partition of the analyte from the mobile phase into the stationary phase and vice versa. The plate number can be directly calculated from the retention time and standard deviation of a normal Gaussian peak as shown in Equation 7. Figure 2

illustrates the standard deviation of a normal Gaussian peak through the band broadening process occurring during separation. A smaller standard deviation, or the narrower the peak, results in an increase in the number of theoretical plates and thus to a more efficient separation [28].

Band-broadening is a general term used to describe the overall dispersion or widening of a sample peak as it passes through a separation system. During the transport of the solute through the column, various mixing processes resulted in for the width of a chromatographic peak broadening [24]. Band-broadening in Chromatography is a result of several effects. These include diffusion processes, transfer of solutes between the mobile and stationary phases, extra-column band broadening.

An important concept in the studies of chromatographic efficiency is the plate height, *H*, which is a measure of how efficient a column is relative to its length. The plate height is defined by Equation 13.

$$H = \frac{L}{N} \tag{13}$$

It follows from the equation that *N* is proportional to the column length and that *H* and *N* are inversely proportional. *H* is therefore a parameter that should be minimized to achieve maximum efficiency.

The main factors contributing to peak broadening have been described by the rate theory [29]. It views the separation process in a packed chromatographic column as a dynamic process of independent mass transfer and diffusion processes that cause band broadening. Molecular diffusion is the moment of molecules from the regions of high concentration to regions of low concentration until the concentration difference is balanced. The rate of this movement is directly proportional to the concentration gradient and in binary systems is expressed as diffusion coefficient D_M (m^2/s).

The so-called van Deemter equation describes the relation of the height of a theoretical plate, H and the average linear velocity of the mobile phase. In condensed form is expressed as follows:

$$H = A + \frac{B}{u} + Cu \tag{14}$$

Where *H* is plate height, *u* is the mobile phase velocity, and the parameters *A*, *B* and *C* are described below.

- A Term (Eddy diffusion)

  Eddy diffusion is the effect in which the flow of molecules in different channel through packed column with different flow paths around the particles resulting in different pathway lengths and consequently broader peaks. Irregularity shape,

particle size and shape are the important properties of packed column. The higher the diameter and irregularity of the particles the stronger is the dispersion. Consequently, the A term can be minimized using small regular particles and a uniform column packing, but at the cost of a higher backpressure.

- B Term

  The B term is directly proportional to the diffusion coefficient D_G of the analytes in the mobile phase. The molecular diffusion overlays the solute transport along the column caused by the pressure drop. The diffusion is caused by concentration differences in the solute band. The effect of the B term is inversely proportional to the carrier gas velocity.

- C term (mass transfer)

  The C terms refers to the mass transfer between stationary and mobile phase. It is also termed resistance against the mass transport. Chromatography is a dynamic process. The effect of the C term linearly increases with the carrier gas velocity. The transport of the solutes into the liquid stationary phase and back to the phase interface are determined by axial diffusion (perpendicular to the flow direction of the mobile phase). Therefore, the C term is determined by the diffusion coefficients of the solute in mobile and stationary phase and the transport distances, most importantly the thickness of the liquid stationary phase [30]. The sum of the effects from B and C terms depend on the mobile phase velocity in addition the values of *A*, *B* and *C*. A typical plot showing the contribution to plate height from the different terms is shown in the van Deemter plot (fig.4). The sums of the three terms has a minimum value at the optimal mobile phase velocity, u_{opt}. The optimal velocity is found where the partial derivative of the van Deemter equation with respect to mobile phase velocity is zero [22], which gives a simple equation for finding u_{opt} from the B and C terms (Equation 15).

$$u_{opt} = \sqrt{\frac{B}{C}} \qquad (15)$$

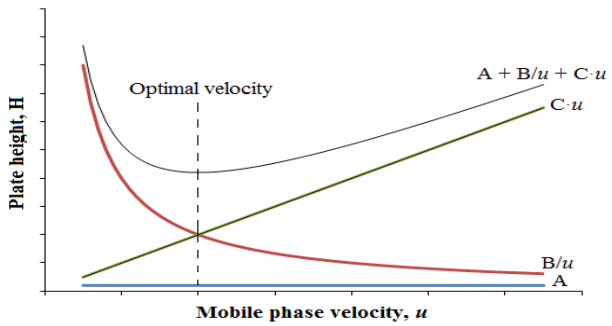

Figure 4: The van Deemter curve

2.1.2 Band Broadening in Capillary Columns: Golay Equation

Capillary gas chromatography (GLC) has been introduced by Golay in 1958 [31]. Unlike the situation with packed column, the equation of the height equivalent to one theoretical plate (HETP), does not include an A term because these columns do not contain a particulate packing material. Band broadening in capillary columns are therefore described by the Golay equation, its simplest form are given by Equation 16.

$$H = \frac{B}{u} + Cu \tag{16}$$

It is common to split the C term into the contribution from the stationary phase, C_S, and the contribution from the mobile phase, C_M (Equation 17).

$$H = \frac{B}{u} + C_S u + C_M u \tag{17}$$

There exists an expanded version of Equation 17 that tells which factors that will influence the plate height in a capillary column (Equation 18)

$$H = \frac{2D_M}{u} + \frac{qkd_f^2}{(1+k)^2 D_S} u + \frac{(1+6k+11k^2)d_c^2}{96(1+k)^2 D_M} u \tag{18}$$

D_M and D_S are diffusion in the mobile and stationary phase, respectively, d_f is the film thickness, d_c is the column diameter, k is the retention factor and q is a quality parameter related to the stationary phase. It can be seen that the C_S term is proportional to the squared film thickness, and that the C_M term is proportional to the squared column diameter. A column with small dimensions therefore have much more plates per meter than a column with large dimensions. The B term is proportional to the diffusion in the carrier gas, and the C_M term is inversely proportional to the diffusion in the carrier gas, which cause large differences u_{opt} for different carrier gases (Equation 15). Hydrogen (high D_M) will for instance have much higher u_{opt} than Nitrogen (low D_M). The C_S term is inversely proportional to the diffusion in the stationary phase, but this can only be manipulated by the temperature and the type of stationary phase.

A challenge when studying plate height in capillary columns is the mobile phase (carrier gas) is compressible, which lead to higher density in the beginning of the column, where the pressure is high, than at the end, where the pressure is low. Because of the difference in density, D_M will be gradually increasing from the injector to the detector. Because of this, Equations 16 to 18 will only fit well to experimental data when the pressure drop is low (short wide bore columns).

Equations 19 and 20 [32] have therefore been introduced to fit data in situations with high pressure drop equation 19.

$$H = \frac{B}{u^2} + Cu^2 \tag{19}$$

$$H = \frac{B}{u^2} + C_1 u^2 + C_2 u \quad (20)$$

In this work, a variant of Equation 19, where the squared u is replaced by u^x, is evaluated (Equation 21).

$$H = \frac{B}{u^x} + C u^x \quad (21)$$

2.1.3 Extra column effects

In addition to the band broadening in the column, explained by the above equations, band broadening can happen outside of the column, i.e. by injection and in the detector. Important factors that determines the extra column effects are injected volume, radius and length of connector tubing, the detector make up gas, and column connections [6, 33]. Sternberg [34] showed the sum of band broadening that has been caused by column effects and extra column effects follows ordinary error propagation and can be expressed by Equation 22.

$$\sigma^2_{total} = \sigma^2_{column} + \sigma^2_{ext} \quad (22)$$

In any study of column efficiency it is critical that the extra column effects are minimized.

2.1.3.1 Sampling rate of data acquisition

The separated components are detected by the detector. Detector provides us with a signal that is generated by the elute passing through it. The electric signal originating in the detector is an analogue signal, but the computer can only deal with digital data so that an analogue to digital converter is used to generate the computer compatible signal.

The detector output is sampled a certain number of times per second, generating data pairs of time and signal values and then stored and form the basis for the electronic data handling. The sampling rate of the analogue signal to digital is so important for band broadening, increasing of peak width, of chromatogram peaks. The smaller sampling rate, the more distorted peak representation will be Figure 5. Asymmetric peaks can be corrected by taking more data points per unit time; in fact, sampling of lots of data can cost us to collect tremendous amount of data size that be able to make busy the computer and that demands a large computer storage space and it increases the time of all post-run computational work without a corresponding gain in analytical quality [24, 34].

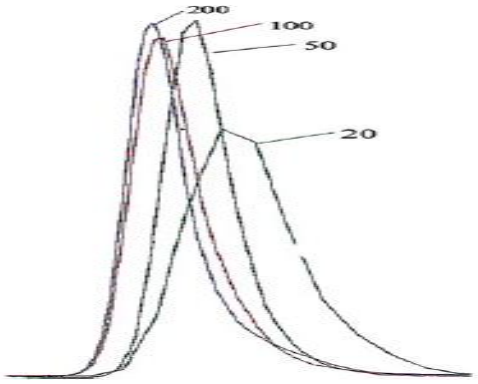

Figure 5 : Effect of data point collection rate on the peak shape at 20, 50, 100, and 200 Hz **[24]**.

2.1.4 Peak capacity (n_p)

According to Giddings' [35] definition, peak capacity is the upper limit of resolving the components for a given techniques under specified working condition. He had also developed a number of mathematical expressions for the peak capacity. The peak capacity over a certain retention range is one such promising criterion as it gives the number of peaks separable with a resolution of unity between two given compounds. Peak capacity and other chromatographic parameters has been illustrated by the following mathematical equation which was suggested by Gidding [36] in collaboration with other scholars (Equation 23).

$$n_{\mathrm{p}} = 1 + \frac{\sqrt{N}}{4}(\ln\frac{t_{\mathrm{R}}}{t_{\mathrm{M}}}) \qquad (23)$$

Where n_{p} is peak capacity and t_{R} is the retention time of the last peak.

Some factors influencing the peak capacity

Peak capacity is exactly means of decreasing the peak width of the chromatogram, the smaller peak width, the more peaks will fit a given separation time. Both in column and off-column (injection, detection, column connection, electronics) are important contributors for the broadening of peak width; this is consequent brings for the lowering of peak capacity [6].

- Mobile Phase Velocity

  At the optimum carrier velocity, u_{0Pt}, the plate number will become high and resulted in giving of maximum number of resolvable peaks occurs at the minimum of the van Deemter plot.

- Column Length

  At a particular temperature and mobile phase velocity, it is believed that the number of plates will increase linearly with the length. For a very long column

length, the value of both HETP and plate number are increasing but the increment of HETP is not as much as plate number. As the column length increases, the plate number will increase and thus improve the peak capacity.

- Temperature

  Harris and Habgood [37] have explored how to elaborate the influence of temperature. The diffusion and partition coefficient of the components in both mobile and stationary phase tell us the impact of temperature on *N*. Frequently, however, the plate number at optimum velocity of the mobile phase initially will increase with rising temperatures and then will decrease with further elevation of the operating temperature [37]. As long as the plate number increases, the peak capacity of the column chromatographic system also increases [37].

- Thickness of Stationary Phase

  Irrespective of column types (packed, capillary) in Gas chromatography, changing film thickness of the stationary phase will affect both the value of plate number and retention factor. The peak capacity of a chromatographic column will decrease when both the volume of stationary phase and film thickness of stationary phase are getting high.

2.1.5 Retention index (RI)

Gas Chromatography is a very widely and powerful techniques to separate exhaustive complex mixture; it is typically used for quantification purpose from the response, peak area, of the analyte and identification of the analyte using retention data including retention index [3]. In 1958, E. Kovats [24] introduced the idea of retention index and equation for determination of it in isothermal chromatography condition using the n-alkanes as reference system. By definition, the retention index of the n-paraffin is equal to 100 times their carbon number regardless of column dimension, stationary phase, temperature, and at any given column condition. In the homologous series elutes retention index is increasing exponentially with retention times, for isothermal GC conditions,.

Equation 24 is being employed in isothermal GC to calculate the retention index of a compound using n-alkanes as reference series.

$$KI = 100\left(\frac{\log t'_{\mathrm{R(x)}} - \log t'_{R(z)}}{\log t'_{\mathrm{R(z+1)}} - \log t'_{\mathrm{R(z)}}}\right) + 100z \qquad (24)$$

where $t'_{\mathrm{R(x)}}$ is the adjusted retention time of compound x (the compound of interest), $t'_{\mathrm{R(z)}}$ is the adjusted retention time of the nearest n-alkane eluting before compound x, $t'_{\mathrm{R(z+1)}}$ is the adjusted retention time of the nearest n-alkane eluting after compound x, and z is the number of carbons in the nearest n-alkane eluting before

compound x [38]. The retention index can also be determined graphically by plotting the log of the adjusted retention time of the n-alkanes against their retention index (fig. ***6***) [21].

Several alternatives to the Kovats indexes exist, and the most common alternative is equivalent chain lengths (ECL). In principle, the ECL system is identical to the Kovats system, but the values assigned to the reference compounds are the number of carbons in the chain length, instead of the number of carbons, times 1. Thus, the ECL values at isothermal conditions are calculated by equation 25 .

$$ECL = \left(\frac{\log t'_{\mathrm{R(x)}} - \log t'_{R(z)}}{\log t'_{\mathrm{R(z+1)}} - \log t'_{\mathrm{R(z)}}}\right) + z \qquad (25)$$

The ECL system has basically been used with fatty acid methyl esters, but can be used with any homologous series as reference compounds.

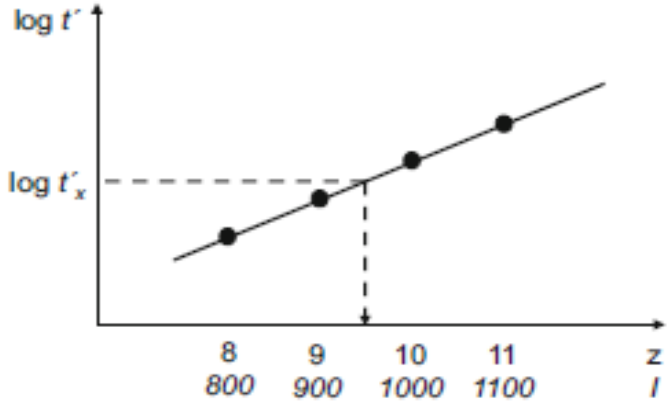

Figure 6: *Graphical determination of Kovats retention indices by plotting the log of the adjusted retention time of the n-alkanes against their retention index* **[21].**

2.1.6 Programmed temperature Gas Chromatography

Temperature programming involves changing temperature in the GC oven rate with either linear or non-linear rate during the chromatographic analysis. It is the method that is suitable for the analysis of samples containing analytes with a wide boiling point range. In temperature-programmed operation, the carrier gas velocity has less importance for resolution and analysis time than in isothermal GC. Rather the ramp rate of the temperature strongly affect the degree of separation and required time to resolve the analytes [10].The temperature programmable operation is advantageous as it allows: higher sample throughput, less carryover, extended application range on a single column, sharper peaks for late eluting components (fig.7). The mathematical relationship of elution temperature with initial column temperature and ramp rate along with analysis time had been reported by Habgood and Harris [10], and are given as Equation 26 and 27 for linear and multiple ramp rates, respectively.

$$T_E = T_S + rt_R \quad (26)$$

$$T_E = T_S + r\,(t_R - rt_{Ri}) \quad (27)$$

Where T_E is elution temperature (°C) of solute, T_S is initial temperature (°C) of the temperature program, r is Heating rate (°C/min), t_R is the last eluted solute retention time of compound, and t_{Ri} is initial isothermal period. Elution temperature, T_E, of a solute is the column temperature in PTGC at which the solute leaves the column.

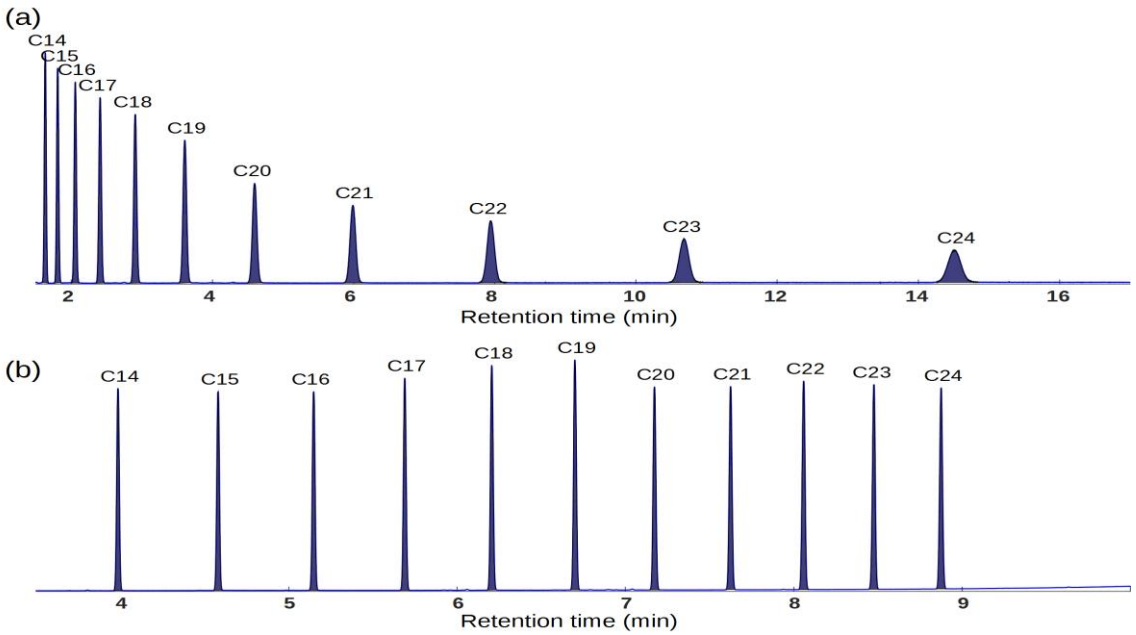

Figure 7: The same C14 to C24 n-alkane mixture analyzed under (a) isothermal and b) temperature-programmed conditions (data from this work).

2.1.6.1 Retention indexes in temperature-programmed gas chromatography

As shown in fig. 6, the relationship between retention time and carbon number is not logarithmic in temperature-programmed GC. Equations 24 and 25 are therefore not valid. However, the concept of retention indices can still be applied. H. van den Dool and D. J. Kratz [39] had proposed an equation for retention index of solute in linear programmed-temperature Gas Chromatography, which does not use the logarithmic form, as presented in Equation 28.

$$KI = 100\left(\frac{t_{R(x)} - t_{R(z)}}{t_{R(z+1)} - t_{R(z)}}\right) + 100z \quad (28)$$

The corresponding equation for ECL values is:

$$ECL = \left(\frac{t_{R(x)} - t_{R(z)}}{t_{R(z+1)} - t_{R(z)}}\right) + z \quad (29)$$

2.1.6.2 Efficiency in temperature programmed gas chromatography

The column performance of gas chromatography, in isothermal condition can be studied using resolution, efficiency, plate height, peak width and peak capacity. However, in temperature-programmed GC, the retention factors are not constant, and factors directly or indirectly dependent on *k* cannot be used. As shown in Equation 18, plate height, and thereby plate number, depends on *k*.

A useful concept for multi-component analysis in programmed GC is to evaluate the number of peaks that can be separated with a defined resolution in a given range of the chromatogram or the whole chromatogram. The effective peak number (*EPN*), the separation number (*SN*), and the peak capacity (n_p) can be used.

Separation number is defined as the number of peaks with good enough resolution in between two consecutive n-alkanes with carbon atom number z and z+1 [24]. *SN* is referred as resolved peaks with resolution of 1.18 between two consecutive members of an n-alkane series. Equation 30 is being used to calculate *SN*

$$SN = \frac{t_{R(z+1)} - t_{R(z)}}{w_{h(z+1)} + w_{h(z)}} - 1 \quad (30)$$

where t_R and w_h are respectively retention time and peak width at half height for two neighbouring members of a homologous series, usually n-alkanes. *SN* is not easy to apply in mathematical modelling because the value of zero does not mean zero separation, because the two reference compounds will still be separated [22].

Peaks per carbon atom (PPC) are inferred as the number of separated peaks per compound in homologs series (including the reference peaks), and can be calculated by Equation 31.

$$PPC = \frac{t_{R(z+1)} - t_{R(z)}}{0.5\left(w_{b(z+1)} + w_{b(z)}\right)} \quad (31)$$

SN and PPC has been referred to as efficiency in programmed-temperature GC, but they can also be viewed as local peak capacity. Combined with the difference in retention indexes (selectivity) they can be applied to calculate resolution by equations 32 to 35, in similar way as the Purnell equation (Equation 8) is applied in isothermal GC.

$$R_s = 0.01177 \cdot \Delta KI \cdot (SN + 1) \quad (32)$$

$$R_s = 1.177 \cdot \Delta ECL \cdot (SN + 1) \quad (33)$$

$$R_s = \Delta KI \cdot PPC \cdot 0.01 \quad (34)$$

$$R_s = \Delta ECL \cdot PPC \quad (35)$$

The equations above show that resolution is directly proportional to PPC, but not directly proportional to SN.

The efficiency (SN or PPC) and selectivity (KI or ECL) both depend on the use of a homologous series, which means that data for calculation of retention indexes will always be available if it is possible to calculate SN or PPC. If retention and peak widths are expressed in retention index units instead of retention time units, the numerator in equation 31 is given by definition and is 1 in the case of ECL scale and 100 with the KI scale. This leads to the following simple expression (Equation 36) that can be used to calculate PPC from any peak with measured on a retention index scale:

$$PPC = \frac{1}{w_{b,ECL}} = \frac{100}{w_{b,KI}} \quad (36)$$

Thus, efficiency (or local peak capacity) can be referred to as the inverse of the peak with measured in retention index units [22].

3. Chemometrics and experimental Design

Chemometrics is an application of combination of mathematics and science including chemistry that used for experimental planning and data mining to provide maximum information from chemical data [40]. It is wonderful techniques to maximise the efficiency of scientific discovery, to minimise waste and cost. It has drawn the attention of researchers to do smarter experiments that give the most information possible with the fewest experiments. Experimental design is chosen in order to estimate the influence of the different variables on the result. And in which, multivariate data can be fitted to an empirical function, usually linear or quadratic with interaction terms, which can be used to provide information about the system [41].
As International Vocabulary of Metrology (VIM) [42], Design of Experiment and its constituents are defined as follow:

- Experimental design: statistical techniques for planning, conducting, analysing and interpreting data from experiment.
- Experimental domain: the experimental 'area' that is investigated and defined by the variation of the experimental variables
- Factors: experimental variables that can be changed independently, Independent variables same as factors
- Responses: the measured value of the results from experiments (retention time, peak width)
- Residual: the difference between the calculated and the experimental result
- Model: equation that relates a response to factors
- Effect: coefficient of a term in a model

3.1. Response surface methodology (RSM)

1950s was the time for the appearing of Response surface methodology (RSM), to the business work by G.E.P. Box and his colleagues [43]. Response surface methodology is statistical and mathematical techniques used to develop, improve and optimize processes in accordance with the predetermined plan by varying the values of factor variables. It is very crucial to determine an optimum and help to illustrate graphically the relation between various level of experimental variables and their responses. Polynomial function contains quadratic model is necessary to be able to determine an optimum value of the variables and response [41]. Equation 37 shows a typical quadratic response model developed from two experimental variables.

$$\hat{y} = b_0 + b_1 x_1 + b_2 x_2 + b_{12} x_1 x_2 + b_{11} x_1^2 + b_{22} x_2^2 \quad (37)$$

Where x_1 and x_2 are representing variable 1 and 2 respectively. The regression coefficients b_0, b_1, b_2, b_{12}, b_{11} and b_{22} is the model and explain how the response (y) depends on the constant, mean value (b_0), the independent variables, main effect (b_1 and b_2), interactions between the independent variables (b_{12}) and the squared independent variables (b_{11} and b_{22}) [42].

3.1.1. Response surface models of retention time and efficiency in PTGC

According to the work of Mjøs and Waktola [22], in a temperature programmed GC, peak width in retention index unit (inverse of efficiency, Equation 36) can be expressed by an expanded van Deemter equation that take into account the influence of the temperature rate in addition to the effect of the carrier gas velocity, Equation 38.

$$w_{b,RI} = a + \frac{b}{u} + cu + di + e\frac{i}{u} + fiu \quad (38)$$

where $w_{b,RI}$, is peak with in retention index units (inverse of PPC by Equation 36). The parameters *a*, *b* and *c* correspond to *A*, *B* and *C* in the van Deemter equation (Equation 14) and the parameters d, e and f are the same terms multiplied with the temperature rate, *i*.

The optimal carrier gas velocity at any temperature rate can be found from the partial derivative of Equation 38 with respect to *u*, in the similar way as for the original van Deemter equation (Equations 14 and 15), and is given by equation 39.14

$$u_{\text{opt}} = \sqrt{\frac{b + ei}{c + fi}} \quad (39)$$

From the same experiments used to resolve Equation 38 by regression, one can find functions that explain the retention time of the last compound by finding the regression coefficients *D*, *E*, *F* and *G* in Equation 40.

$$\ln(t_{\text{R}}) = D + E\ln(u) + F\ln(i) + G\ln(u)\ln(i) \quad (40)$$

Equations 38 and 40 are used to model efficiency and retention time of the last compound to study the balance between achieved efficiency and how long time it will take the last compound in temperature programmed GC [22].

Figure 8 a and b, shows response surface models based on Equation 38 and 40, respectively. The grey line in figure 8a represents the optimal velocities (u_{opt}) calculated by Equation 39. In figure 8c, the most important iso-lines from figure 8a are overlaid on the response surface for the retention time model (Figure 8b). The black line next to the white u_{opt} line show the conditions that will minimize the time required to achieve a certain efficiency (defined as PPC).

3.1.2. Evaluation of model fitness

The error of models and its fitness is evaluated by following statistical measure [41].

1. The values of coefficient of determination or explained variation, R^2, and
2. Root means squared error (RMSE).

They are calculated as:

$$\boldsymbol{R^2 = (1 - \sum_i^n \frac{(\hat{y}i - yi)^2}{(yi - \bar{y})^2})} \quad (41)$$

$$RMSE = \sqrt{\sum_{i=1}^{n} \frac{(\hat{y}i - yi)^2}{n}} \quad (42)$$

$\sum(\hat{y}i - \bar{y})^2$ is the sum of squares residuals.

$\sum(yi - \bar{y})^2$ is the "total sum of squares" and quantifies how much the data points, y_i vary around their mean.

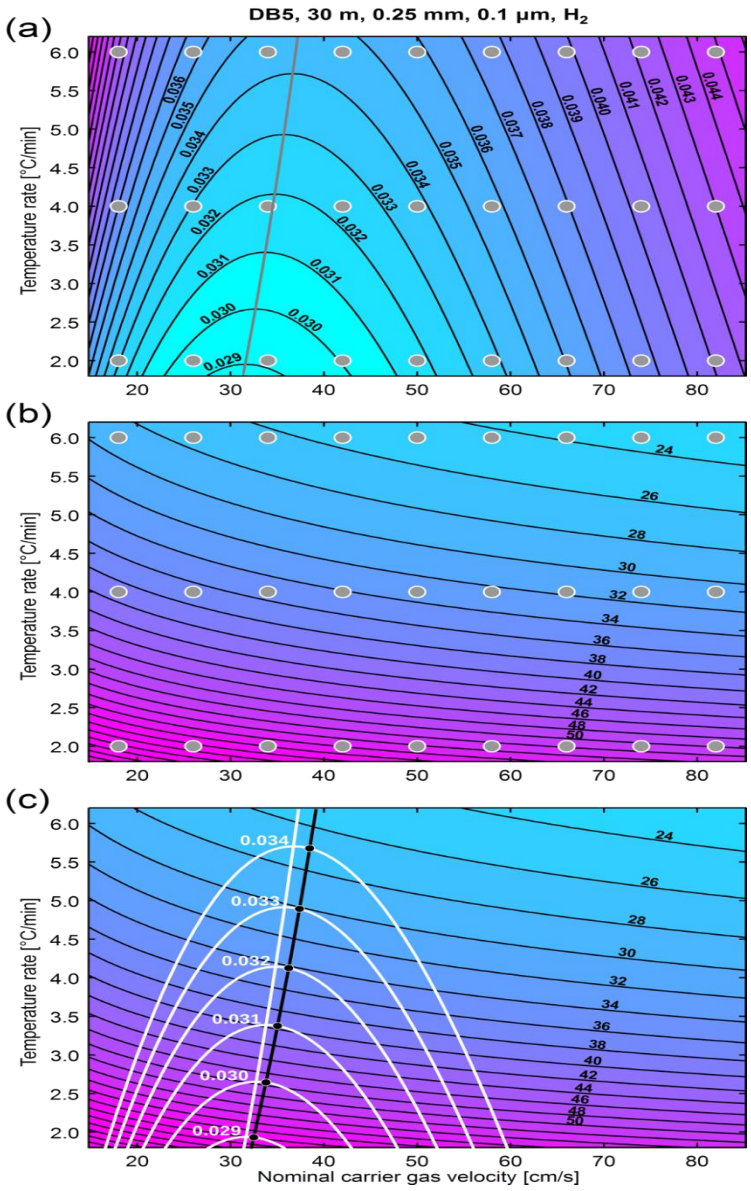

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Figure 8: surface response plot a) VD+Int b) logt_R c) efficiency –time adapted from [22].

3.2 Design of Experiments (DoE)

Sample preparation, determining experimental condition, qualitative identification, and quantitative determination are the usual activities in chromatographic analytical methods. Of these, the first two, Sample preparation and determining experimental condition have been frequently optimized using multivariate Chemometrics techniques [44].

Multivariate statistical methods require experimental domain of the factor minimum and maximum values to be investigated during the optimization procedure. The combinations of the different factor levels used to perform the actual experiments are then decided by which multivariate technique is employed [22]. The most commonly used designs to determine response surfaces are the factorial designs, central composite, Box–Behnken, Doehlert, and user defined design.

3.2.1 Factorial Design

Factorial designs are used to study the influences of all experimental variables, factors, and interaction effects on the response and widely employed for screening purpose to investigate main and interaction effects. For **k** number of factors at **L** levels, $\mathbf{L^k}$ numbers of experiments are going to be conducted. At two levels only four experiments are needed for two factors while eight experiments should be carried out for three factors. The number of experiments increases drastically as the number of levels increase [41].

3.2.2 Central composite Design

It is the expanded form of factorial designs and is the combination of a two-level factorial design and additional axial points and at least one centre points in the design. Central composite designs require $L^k + Lk + nc$ where nc, L and k are the number of replicate centre points, variable level and number of variables respectively. The factorial points will contribute in estimating the interaction terms and the axial points will contribute in estimating the quadratic terms [42]. Although the factorial designs can be used to determine simple response surfaces that are linear in all of the investigated factors, they are normally used to determine which experimental factors are the most important to investigate and which factors do not significantly affect the experimental results.

3.2.3 User defined design

Unlike the other experimental design, in user defined design the numbers of experiments are flexible and limited by the interest of the user [45]. This design is applied in our entire experimental activities with two factors that contains 25 and 5 level carrier gas (helium, hydrogen and nitrogen) & temperature gradient respectively, for temperature programmed GC; while factors in isothermal condition the factor is velocity of carrier gases with ten levels to be investigated in the study.

4. Experimental

4.1 General description

Unless stated otherwise, all experiments were conducted under the following conditions. All analyses were performed using the same Agilent 7890A gas chromatograph, which was capable of using three different carrier gases (helium, hydrogen and nitrogen). The chromatograph was equipped with split/splitless injector, electronic pressure control, Agilent 7683B auto sampler and FID detector. The GC systems were controlled by Agilent Chemstation B.04.03. A 5 µL syringe size was used to inject 0.5 µL of FAMEs and alkanes to the injection port. The injection mode used was split injection with split ratio of 1:200 at 250°C. A pre and post wash of the injection needle were performed using methanol and isooctane. The FID detector was heated at a temperature of 325°C and the flows of the carrier gas, air and make up gas were at 40, 400 and 40 mL/min respectively. The purity of all gases was 99.999%. All experiments with temperature-programming and isothermal were performed in constant flow mode, which means that the mass flow of carrier gas from the column was constant throughout the chromatographic run. Because of gas expansion as the oven temperature increases, in reality the carrier gas velocity continuously increases. Thus the term “nominal carrier gas velocity” refers to the estimated average velocity at injection temperature (60°C), assuming that actual column dimensions were identical to nominal dimensions. All velocities in the results part refer to the nominal average velocities, and these were estimated by the built-in algorithm in the chromatographs.

4.2 Capillary columns

Column used in this work is capillary columns that has been manufactured by Agilent or Quadrex. All columns had 0.25 mm diameter and 0.25 µm phase thickness, which gives a phase ratio of 250.

The stationary phases are 5% phenyl 95% dimethylpolysiloxanes, which is a non-polar stationary phase, low column bleeding with maximum temperature limitations of 350°C. These phases are employed for compounds like Semi-volatiles, alkaloids, drugs, FAMEs, aromatic, hydrocarbons, waxes, flavours, halogenated, pesticides, herbicides [46, 47]. An overview is the columns is given in Table **1**. Column number 1-3 was used for initial testing and pilot studies, while the main experiments were performed on column 4.

Table 1. Column description

Column number	Column type	L(m)	Comments
1	DB-5(Agilent)	10	Used column with unknown history. Prepared by cutting of the first and last 10 m sections from a 30 m column.
2	HP-5 (Agilent)	30	Column previously used for pure standards, known to be in perfect order.
3	DB-5 (Agilent)	60	Used column with unknown history.
4	007-5 (Quadrex)	10,20, 30, 40, 50, 60	Bought as new 60 m column. The length was gradually reduced by cutting off 10 m sections.

4.3 Samples

The analytes used in the study were n-alkanes (C14-C24) or fatty acid methyl esters (FAME). Alkane and FAME samples were diluted from 4.4 mg/ml to 0.4 mg/ml in isooctane in two different mixtures, one containing all the alkanes and one containing all the FAMEs. Isooctane were of chromatography quality (>99% purity) and purchased from Sigma Aldrich. An overview of the content in the samples are given Table 2.

Table 2. samples used in the entire project

Sample 1, Alkanes			**Sample 2, FAMEs**		
name	Purity (%)	orgin	name	Purity (%)	Origin
C14	99.3	Chem Service (West Chester, PA, USA	12:0	99	Nu-Chek Prep (Elysian, MN, USA
C15	99.5	Chem Service (West Chester, PA, USA	12:0	99	Nu-Chek Prep (Elysian, MN, USA
C16	99	Chem Service (West Chester, PA, USA	14:0	99	Nu-Chek Prep (Elysian, MN, USA
C17	99.5	Chem Service (West Chester, PA, USA	15:0	99	Nu-Chek Prep (Elysian, MN, USA
C18	99	Chem Service (West Chester, PA, USA	16:0	99	Nu-Chek Prep (Elysian, MN, USA
C19	99.6	Chem Service (West Chester, PA, USA	17:0	99	Nu-Chek Prep (Elysian, MN, USA
C20	99	Chem Service (West Chester, PA, USA	18:0	99	Nu-Chek Prep (Elysian, MN, USA
C21	99.5	Sigma Aldrich (St Louis, MO, USA)	19:0	99	Nu-Chek Prep (Elysian, MN, USA
C22	98	Fluka (Buchs, Switzerland)	20:0	99	Nu-Chek Prep (Elysian, MN, USA
C23	99.5	Sigma Aldrich (St Louis, MO, USA)	18:3 n-6	99	Nu-Chek Prep (Elysian, MN, USA
C24	99.5	Sigma Aldrich (St Louis, MO, USA)	20:3 n-6	99	Nu-Chek Prep (Elysian, MN, USA

4.4 Chromatographic conditions

The conducted experiments can be divided into 6 categories:

1. Initial experiments and study of extra column effects
2. Temperature programmed pilot studies
3. Isothermal pilot studies
4. Main temperature-programmed studies
5. Main isothermal studies
6. Final quality check of the column

The various experiments are numbered. In general, the numbering reflects the sequence the experiments were conducted in, not the topics of the different experiments.

4.4.1 Initial experiments and study of extra column effects

All these experiments were carried out on column 1 and, with one exception, helium as carrier gas. Only Sample 1 (alkanes) was applied. The main purpose of the study was to study extra-column effects and provide data necessary to design the subsequent experiments. The purpose and the experimental conditions for these experiments are given below:

Test of split ratio (experiment 1 and 2)

- Purpose: to investigate the effect of injector split ratio on peak widths
- Principle: The sample was injected with different split ratios (1:200, 1:100, 1:50, 1:25 1:12.5, and 1:6.25). The sample dilution (1:1, 1:2, 1:4, 1:8, 1:16, 1:32) was adjusted according to the split ratio so that the total dilution was 1:200. Detector makeup gas was set to the maximum limit for the instrument (60 psi) to avoid peak broadening in the detector.
- Temperature programmed conditions (experiment 1): injection at 60°C, 30°C/min to 270°C, and nominal carrier gas velocity was 30 cm/s.
- Isothermal conditions (experiment 2): Oven temperature was set to 200°C and the program was run for 14 min.

Check of effect of detector makeup gas (experiment 3 and 4)

- Purpose: to investigate the effect of detector makeup gas on peak widths
- Principle: The makeup gas flow was varied from 0 to 60 ml/min in steps of 10.
- Temperature programmed conditions (experiment 3): Same as experiment 1 with split ratio set to 1:200.
- Isothermal conditions (experiment 4): Same as experiment 2 with split ratio set to 1:200.

Check of the effect of start temperature (experiment 5)

- Purpose: To investigate the effect of start temperature in the temperature-programmed experiments
- Principle: Start temperature was varied from 40 to 160°C in steps of 20. The test was conducted with low (5°C/min) and high (25°C/min) temperature rates
- Other conditions: Injector split ratio: 200:1, nominal carrier gas velocity: 25 cm/s, Program end temperature: 280°C.

Retention at different temperatures under isothermal conditions (experiment 6)

- Purpose: to determine retention factors of the alkanes at different temperatures
- Principle: the oven temperature was varied from 160 to 230°C in steps of 10.
- Other conditions: Injector split ratio: 200:1, carrier gas velocity: 25 cm/s

Test of limits for column overload (experiment 7)

- Purpose: to investigate how large mass of each compound that could be injected without seeing column overload.
- Principle: Solutions of 12.5, 25, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 ng/µl of each of the alkanes was analyzed with an injection volume of 0.5 µl and a split ratio of 1:40, which means that the amounts applied to the column ranged from 0.16 to 6.3ng. The experiments was conducted with low (5°C/min) and high (25°C/min) temperature rates
- Other conditions: Same as described for experiment 5, with split ratio of 1:40 and start temperature of 60°C

Test of effects of detector frequency (experiment 14)

- Purpose: to investigate if and how detector frequency had an effect on peak widths
- Principle: the alkanes were analysed with varying detector frequencies (10, 20, 50 and 100 Hz). Each condition was replicated six times.
- Temperature-programmed conditions: Injection at 60°C, 25°C/min to 270°C. Hydrogen was used as carrier gas with a nominal velocity of 78 cm/s. Split ratio was 1:200.

4.4.2 Programmed temperature pilot studies

The main purpose of the project was to investigate how carrier gas velocity and temperature rate affects retention times and peak capacity in temperature programmed GC. Before the main experiments, pilot studies on three columns (column 1-3) with different length were conducted.

Carrier gas velocity and temperature rates was varied according to the skewed plot (fig.9) proposed by Mjøs and Waktola [22], with five levels of temperature rate and 25 levels of carrier gas velocity. The levels of temperature rate are replicated as shown in figure 9, while the levels of carrier gas velocity are unique.

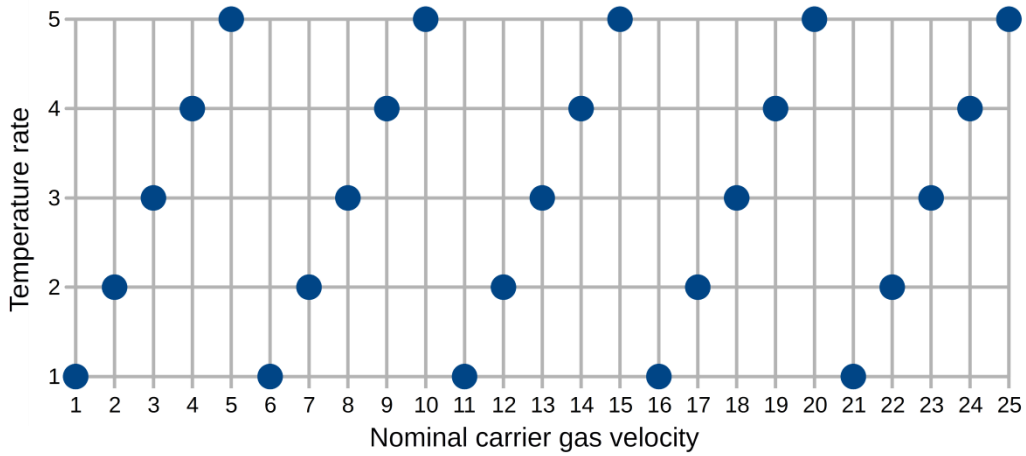

Figure 9: illustration of the design used in temperature-programmed experiments.

The levels of the two parameters are linearly spaced, which means that each variable can be described by start: rate: end. The conditions for the different pilot experiments are reported in

Table 3. The levels of the product of temperature rate and column length (ratelength) are the same for all column lengths (50, 100, 150, 200 and 250 m°C/min). Selected carrier gas velocities were based on expectations from previous works [22].

Table 3. *Conditions for programmed temperature pilot experiments*

Exp. Number	Carrier gas	Col. Length [m]	Temp. rate [°C/min]	Velocity, *u* [cm/s]
10	H2	10	5:5:25	18:2.5:78
8	He	10	5:5:25	14:1.3:45.2
12	N2	10	5:5:25	8:0.7:24.8
24	H2	30	1.67:1.665:8.33	18:2.5:78
26	He	30	1.67:1.665:8.33	18:1.3:45.2
22	N2	30	1.67:1.665:8.33	8:0.7:24.8
18	H2	60	0.83:0.835:4.17	18:2.5:78
16	He	60	0.83:0.835:4.17	14:1.3:45.2
20	N2	60	0.83:0.835:4.17	8:0.5:20

4.4.3 Isothermal pilot studies

Isothermal pilot studies were conducted at an oven temperature of 210°C. There were nine levels of carrier gas velocity. The conditions are reported in Table 4.

Table 4. Conditions for isothermal pilot experiments

Exp. Number	Carrier gas	Col. Length [m]	Temperature [°C]	Velocity, *u* [cm/s]
11	H2	10	210	20:9:92
9	He	10	210	14:4:46
13	N2	10	210	8:2:24
25	H2	30	210	18:7:74
27	He	30	210	14:4:46
23	N2	30	210	8:2:24
19	H2	60	210	18:7:74
17	He	60	210	14:4:46
21	N2	60	210	8:1.5:20

4.4.4 Main programmed temperature studies

All main studies were conducted on Column 4. This was originally 60 m, but the length was gradually reduced by 10 m cutting off sections of 9.5 m and removing additionally 0.5 m at installation (after the septum, septum nut and ferrule put on to prevent unsafe installation). The same type of design as applied with the pilot studies was used, but it was decided to increase ratelengths to 60, 120, 180, 240 and 300 m°C/min (6/5 of the pilot studies). The carrier gas velocities was set based on the models from the pilot study using the following criteria: predicted peak widths in retention index units ($w_{b,RI}$) in the lower left corner and the lower right corner (ratelength 60) of the design (fig.16) should be approximately equal to the widths at optimal velocity and a ratelength of 300. In addition, the nominal carrier gas velocity should not be below

6 cm/s and it should not have more than one decimal. In some cases (long columns and N_2) the instrument limits also put a constraint on how high carrier gas velocities that could be used. Then a limit for carrier gas velocities for column lengths that was not in the pilot study was found by extrapolation. The applied conditions are reported in Table 5. Both Sample 1 and Sample 2 were analyzed. The different programs (conditions) in an experiment were run in randomized order so as to control uncontrolled variability. Sample 2 was always analyzed immediately after sample 1 (alkane, the retention index of alkane is stable regardless of column condition and used for identification of second sample). A dummy sample was always run in the beginning of the sequence to make free from contaminant.

Table 5. Conditions for temperature-programmed experiments on the 007-column

Exp. Number	Carrier gas	Col. Length [m]	Temp. rate [°C/min]	Velocity, *u* [cm/s]
60	H2	10	6:6:30	17.3:3:89.3
62	He	10	6:6:30	11.7:2.4:69.3
58	N2	10	6:6:30	6:1:30
54	H2	20	3:3:15	16:2.8:83.2
52	He	20	3:3:15	11:2.2:63.8
56	N2	20	3:3:15	6:0.9:27.6
48	H2	30	2:2:10	14.7:2.6:77.1
46	He	30	2:2:10	10.3:2:58.3
50	N2	30	2:2:10	6:0.8:25.2
42	H2	40	1.5:1.5:7.5	13.4:2.4:71
40	He	40	1.5:1.5:7.5	9.6:1.8:52.8
44	N2	40	1.5:1.5:7.5	6:0.7:22.8
36	H2	50	1.2:1.2:6	12.1:2.2:64.9
38	He	50	1.2:1.2:6	8.9:1.6:47.3
34	N2	50	1.2:1.2:6	6:0.6:20.4
30	H2	60	1:1:5	10.8:2:58.8
28	He	60	1:1:5	8.2:1.4:41.8
32	N2	60	1:1:5	6:0.6:20.4

4.4.5 Main isothermal experiments

These experiments were always run after the corresponding (same carrier gas and column length) programmed experiments. The oven temperature (210°C) was the same as in the pilot studies, but it was decided to increase to 10 levels on the carrier gas velocity. The selected levels for the carrier gas velocity was based on the models from the pilot study, and the upper and lower limits was set so that the expected plate height at minimum and maximum velocity was approximately 0.4, but with the same constraints as for the temperature-programmed experiments. The conditions are reported in Table 6.

Table 6. Conditions for isothermal experiments on the 007-column

Exp. Number	Carrier gas	Col. Length [m]	Temperature [°C]	Velocity, *u* [cm/s]
61	H2	10	210	18:11.3:119.7
63	He	10	210	14:8.4:89.6
59	N2	10	210	6:3:33
55	H2	20	210	17.3:10.3:110
53	He	20	210	13.2:7.5:80.7
57	N2	20	210	6:2.8:31.2
49	H2	30	210	16.6:9.3:100.3
47	He	30	210	12.4:6.6:71.8
51	N2	30	210	6:2.6:29.4
43	H2	40	210	15.9:8.3:90.6
41	He	40	210	11.6:5.7:62.9
45	N2	40	210	6:2.4:27.6
37	H2	50	210	15.2:7.3:80.9
39	He	50	210	10.8:4.8:54
35	N2	50	210	6:2.2:25.8
31	H2	60	210	14.5:6.3:71.2
29	He	60	210	10:3.9:45.1
33	N2	60	210	6:2:24

4.4.6 Final quality check of the column

A final quality check of the column was conducted by comparing the first 9.5 m section cut off from the 60 m column with a 9.5 m section cut from the 10 m column that was left after all the main experiments. After installation in the GC, these sections were 9.05 m. The checking of quality of the column was conducted in isothermal mode with helium as carrier gas. Except for the 0.95 m difference in column length the conditions are the same as reported for experiment 63 in Table 6. These are experiment numbers 64 (first section) and 65 (last section).

5. Result and discussion

5.1 Extra column effect

The efficiency of chromatography can be influenced by both extra-column and internal column effects [48]. The effects of these variables on the quality of chromatogram were studied. of these, the starting temperature of the oven, the split ratio of sampling mode, the sampling rate in the detector, the flow rate of detector gas (make up gas) and the amount of sample, that should be loaded to the column, were the explored fundamental parameters for the analysis on peak width and detector sensitivity.

5.1.1 Starting temperature

In programmed Gas chromatography, starting temperature is the vital condition for both column efficiency and time. For the entire work of the analysis, the oven starting temperature at 40, 60, 80, 100, 120, 140 and 160°C was optimized to know the influence for efficiency (peak width, RI). Peak width, in RI unit, of the chromatogram and starting temperature are described in fig. 10. The data, with less in peak width, that have been generated using the starting temperature of 40, 60, and 80°C were chosen. Student's t-test hypothetical testing method has been used to know the significance difference in average peak width with one another (Appendix A, table 14 and 15). With 99.955% and more confidence, there is significance difference between them. 60°C was the best condition for the main works; at 40°C will not have good analyte focusing while at 80°C and above, the diffusion of solvent in to carrier gas streams which brings the retention time of early eluting to un retain.

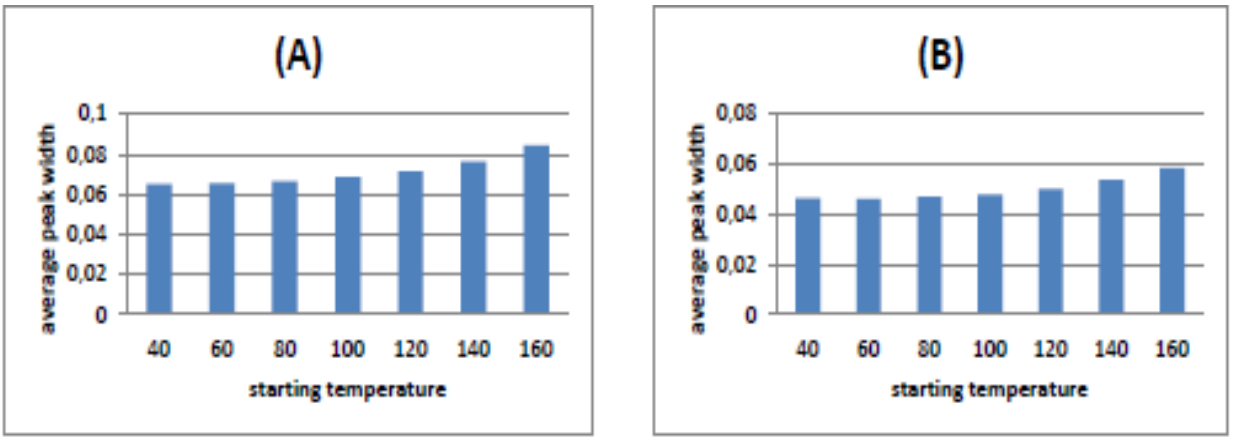

Figure 10 : average peak width at ramp rate (A) 25 ℃/min (B) 05 ℃/min (HP-5, 10 m x 250 μm x 0.25 μm, He carrier gas, constant flow rate.

5.1.2 Split ratio

For both programmed temperature and isothermal oven temperature condition with split/splitless injection mode, the split ratio (1:200, 1:100, 1:50, 1:25, 1:12.5 and 1:6.25) of the inlet sample were studied and optimized. Split ratio has been contributing for band broadening and causing not to have Gaussian peaks [49]. Early eluting analyte chromatogram

is properly focused and their peak width is narrower for split ratio of 1:200 while in the last three split ratios including 1:6.25 the early eluting solutes are not focused and the peak width become broad (fig. *11:*. a, b). All split ratio fractional peak width is calculated in terms of peak width of 1:200 presented in fig. 11:c. As can be seen (Appendix A, table 17), in the three highest splits (1:50, 1:100, and 1:200) that C_{14} and C_{15} were separated from the solvent, and the others are therefore not relevant. The effects of the split are highest on the first peaks since the probabilities of the analytes are being hidden by the solvent is high. For C_{24}, only 1:6.25 showed clear deviations from the other splits. If we compare with the 1:200 split, there is 25% increase in the peak width for C_{14} if we choose a 1:50 split, but only a 6.3% increase if we choose a 1:100 split. The corresponding numbers for the averages are 2.1% and 8.8%. Because of instrument limitations the split ratio cannot be set much higher than 1:200, but based on the small differences between 1:200 and 1:100 we could not expect a significant decrease in the peak widths if we could have a higher split ratio. However, there is a difference between 1:100 and 1:200 that could be high enough to affect the models. Therefore, 1:200 split ratios was decided for further experiments.

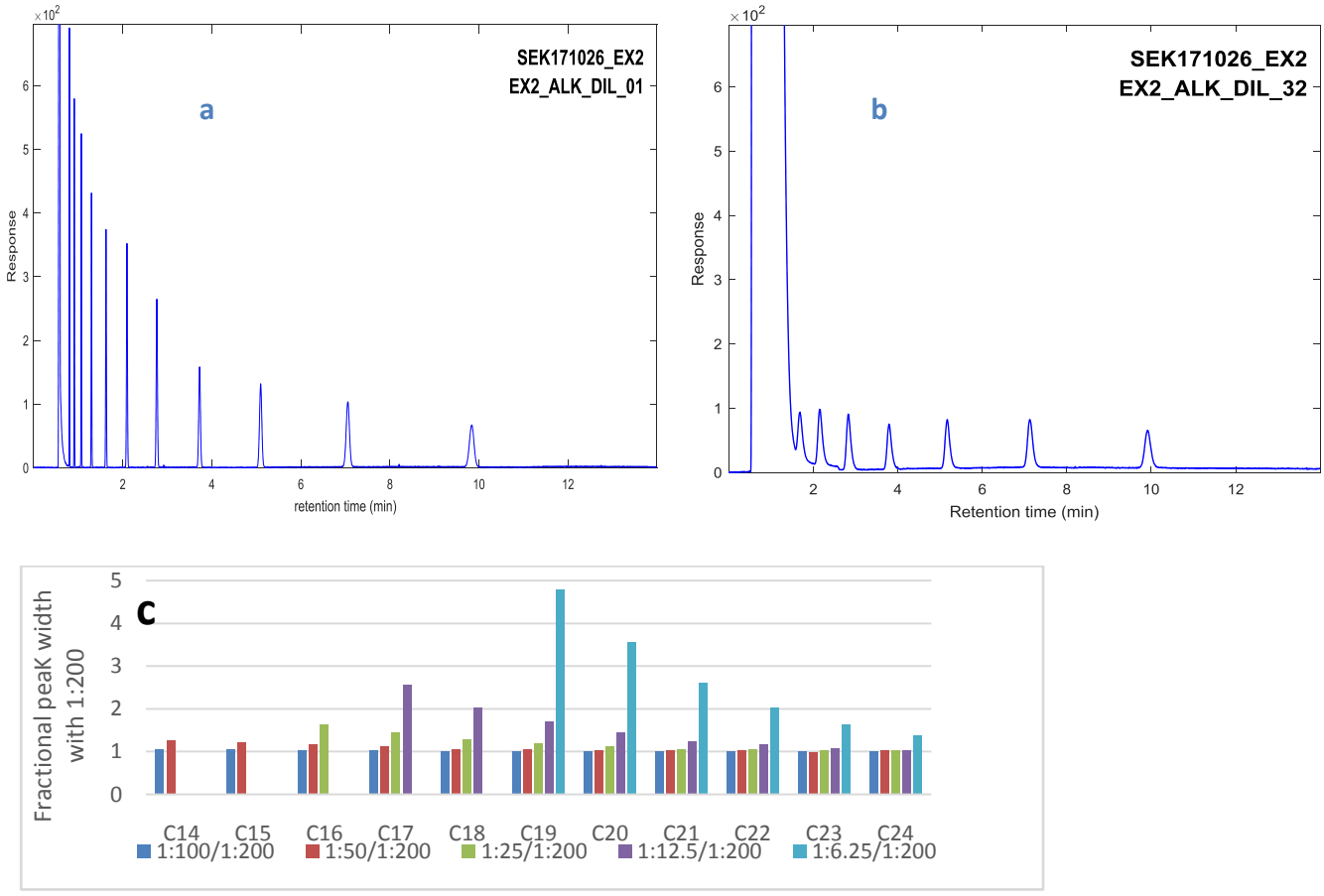

Figure 11: a) Chromatogram of 1:200 split ratio b) Chromatogram of 1:6.5 split ratio c) % of peak width with respect to 1:200 (HP-5,10 m x 250 µm x 0.25 µm, 200 °C isothermally, 30 cm/s, velocity of carrier gas, He, constant flow rate)

5.1.3 Makeup gas flow rate

For both programmed temperature and isothermal oven temperature condition, the flow rates of the detector makeup gas was studied and optimized, fig.12. The efficiency of chromatography can be evaluated by peak width and peak area; the higher the peak width of the chromatogram, the lower efficiency of chromatogram and vice versa as written [50]. And in addition to this, the higher peak area, the better efficiency of column and the detector become more sensitive Peter Koryta, H.-G. J. (2002). Maximum peak area was recorded when the flow rate of the detector make up gas at 40 mL/min for both oven temperature. Since, statistically speaking there was no significance difference between the two condition but the maximum peak area for all makeup gas flow rate were achieved in programmed temperature. So that the preferred detector make up gas flow rate was 40mL/min which obtained from programmed temperature capillary gas chromatography.

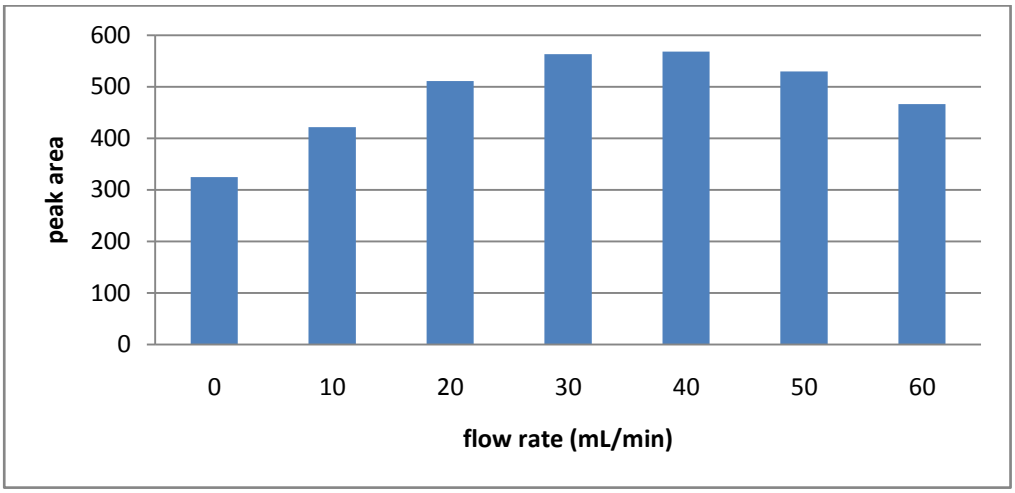

Figure 12: Peak width and Make up gas flow rate (HP-5,10 m x 250 µm x 0.25 µm, 60 °C for 0 min, 30 °C/min, 30 cm/s, velocity of carrier gas, He, constant flow rate, 1:200 split ratio).

5.1.4 Detector frequency

The sampling frequency of data from the detector, converting analogue to digital, has been studied to ready for further study and presented in the sampling rate and peak width of the chromatogram in fig.13. In this work, the four sampling rate were chosen and evaluated their impact on the peak width, RI, of the peak; student's t-test hypothetical testing method has been used to know the significance difference in average peak width with one another (Appendix A table,16). The average peak width in 10 Hz is statistically significance difference in confidence of level of 99.999% with the rest of average peak width of the sampling rate (20 Hz, 50Hz and 100Hz). The three sampling frequency with no significance difference in peak width were the ideal and employed for the entire work; in fact as the sampling rate increase, the peak width of the sample peak decrease. Of course, too big sampling rate can bring a problem of demanding large computer to store the data and bringing to increases the time of all post-run computational work without a corresponding gain in analytical quality.

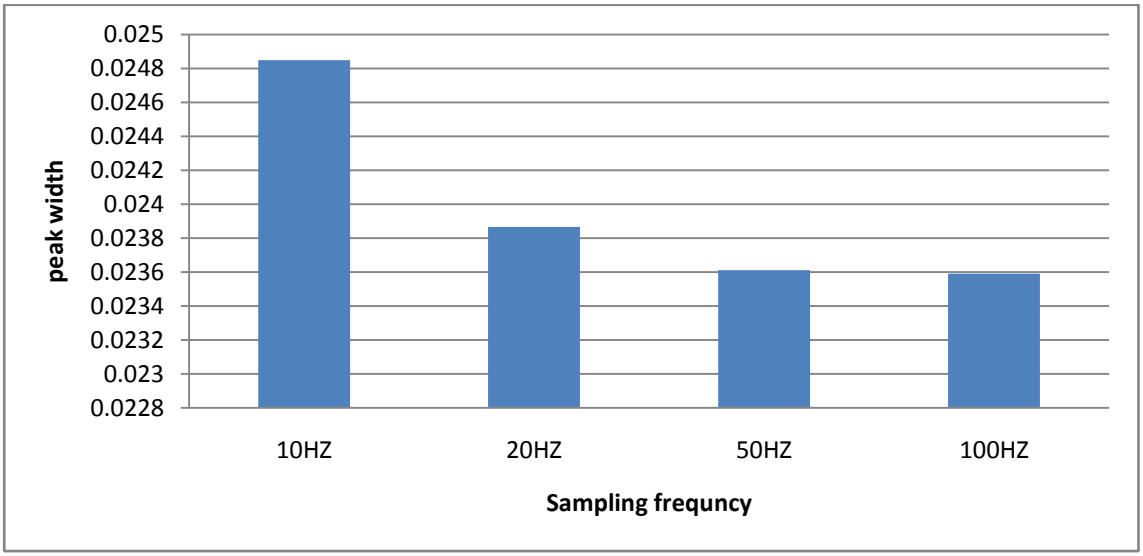

Figure 13: Peak width and detector frequncy (HP-5,10 m x 250 µm x 0.25 µm, 60 °C for 0 min, 30 ℃/min, 30 cm/s, velocity of carrier gas, He, constant flow rate, 1:200 split ratio, 40mL/min make up gas flow rate).

5.1.5 Asymmetry

In chromatography, the degree and nature of peak asymmetry are indicative of problems with stationary phase kinetics, thermodynamics, or extra column effects. Peak asymmetry is the characteristics of peak shape in which the peak is either tailing or fronting depending on the amount of sample injected [51]. In our experiment, programmed temperature at 05 and 25°C/min, the concentration of the sample and its impact on the nature of peak was studied. In each temperature rate, the injected sample concentration; its corresponding peak asymmetry factor and peak width, Rt unit, are presented in (fig.14,Table 7). As shown in the plots, as the concentration of the injected (mass of analyte) sample increased, the peak asymmetry of the peaks getting decreased and also the peak become more fronting since the asymmetry factor goes to less than one. The mass of analyte greater than 1.25ng caused to have lower asymmetry factors.

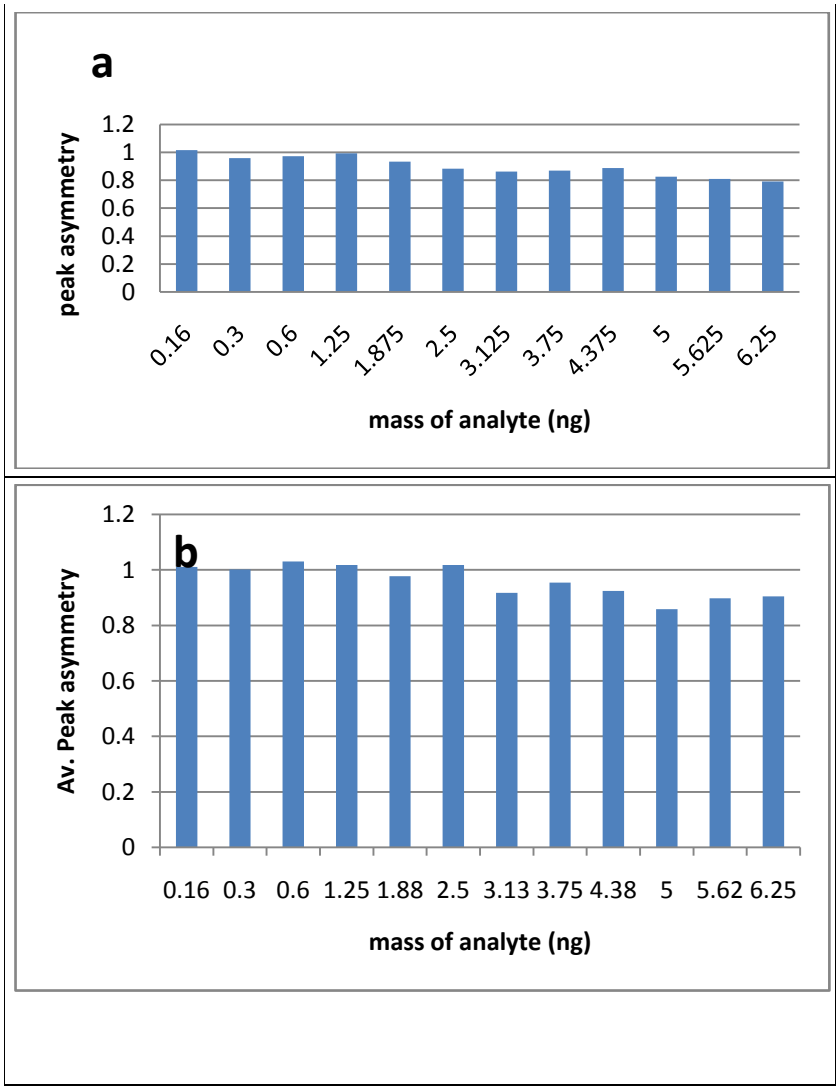

Figure 14: average peak asymmetry at ramp rate (a) 05 ℃/min (b) 25 ℃/min (HP-5,10 m x 250 µm x 0.25 µm, 60 °C for 0 min, 30 cm/s, velocity of carrier gas, He, constant flow rate, 1:40 split ratio)

Table 7. average peak asymmetry at ramp rate 25 ℃/min and 05 ℃/min

Mass of analyte (ng)	05°C/min		25°C/min	
	Average peak width (Rt)	Average assymetry	Average peak width	Average assymetry
0.16	0,0868	1,0168	0,0243	1,0111
0.3	0,0873	0,9588	0,0244	1,0019
0.6	0,087	0,9735	0,0244	1,0305
1.25	0,0866	0,9926	0,0240	1,0177
1.88	0,0861	0,9339	0,0236	0,9767
2.5	0,0859	0,883	0,0243	1,0181
3.13	0,0864	0,8619	0,0241	0,9171
3.75	0,0865	0,8698	0,0237	0,9542
4.38	0,087	0,8878	0,0244	0,9238
5	0,0865	0,8256	0,0241	0,8584
5.63	0,0867	0,8096	0,0238	0,8975

5.2 Retention factor and oven temperature evaluation

Alkane samples were analysed by HP-5 capillary gas chromatography with column 1 and helium as carrier gas, 25cm/s with oven temperature of 160, 230°C, in steps of 10°C between the column temperature. From this experimental condition, for further isothermal study, the last eluted solute retention factor of alkane samples was determined and the data is tabulated in table 8. As equation 8 for isothermal, resolution of chromatogram is the function of selectivity,α, efficiency, N, and retention factor; too low retention factor gives poor resolution and high retention factor it may give us better resolution but very extended analysis time. Scholars had put mathematical formula, that has been derived from equation 8, to calculate optimum retention factor [52]. The optimum average retention factor is the value where $(k+1)^3/k^2$ getting minimum. As shown in fig.15, the optimum average retention factor has been recorded at 210, 220, 230°C. The ideal value of retention factor was the value at 210°C lower than this the analysis required long time higher than this temperature, there would be poor resolution between early eluting peaks.

Table 8. Retention factor obtained from 10m×250µm×0.25µm, He as carrier gas, 25 cm/s, isothermal condition.

Temp	C17	C18	C19	C20	C21	C22	C23	C24	$(k+1)^3/k^2$
160°C	5.527	8.684	13.614	21.301	33.281	51.828	80.463	124.624	34.594
170°C	3.731	5.729	8.776	13.423	20.493	31.194	47.393	71.806	22.032
180°C	2.582	3.876	5.811	8.699	12.993	19.373	28.803	42.771	14.948
190°C	1.828	2.689	3.948	5.789	8.475	12.386	18.067	26.296	10.886
200°C	1.321	1.905	2.746	3.948	5.672	8.132	11.634	16.619	8.558
210°C	0.970	1.376	1.950	2.754	3.881	5.465	7.687	10.791	7.306
220°C	0.726	1.015	1.413	1.963	2.719	3.766	5.204	7.182	6.790
230°C	0.555	0.761	1.042	1.425	1.948	2.652	3.609	4.905	6.854

'k' is the retention factor of the last eluted analyte with their corresponding oven temperature.

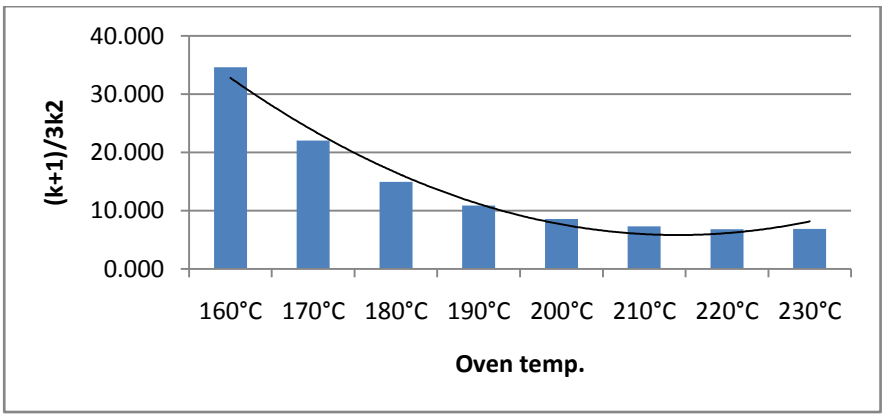

Figure 15: Retention factor obtained from 10m×250µm×0.25µm, He as carrier gas with velocity of 25 cm/s, isothermal condition, 1:200 split ratio, 40 mL/min flow rate of detector gas.

5.3 Pilot study

The main purpose of the pilot experiments, which were conducted on three different column (1-3), used to find suitable instrument settings for the main experiments with column lengths from 10 to 60 m.

The pilot experiments were run with the same carrier gas velocities on all three column lengths, except nitrogen on the 60 m column where the instrument limits did not allow velocities above 20 cm/s. The plots showing optimal conditions are shown in the figure below (fig.16). They show clearly how the optimal carrier gas velocities are highly dependent on the column length and decrease with increasing length. The optimal velocities are far from the center of the x-axis. It was therefore decided to adapt the carrier gas velocities to the column lengths in the main experiments.

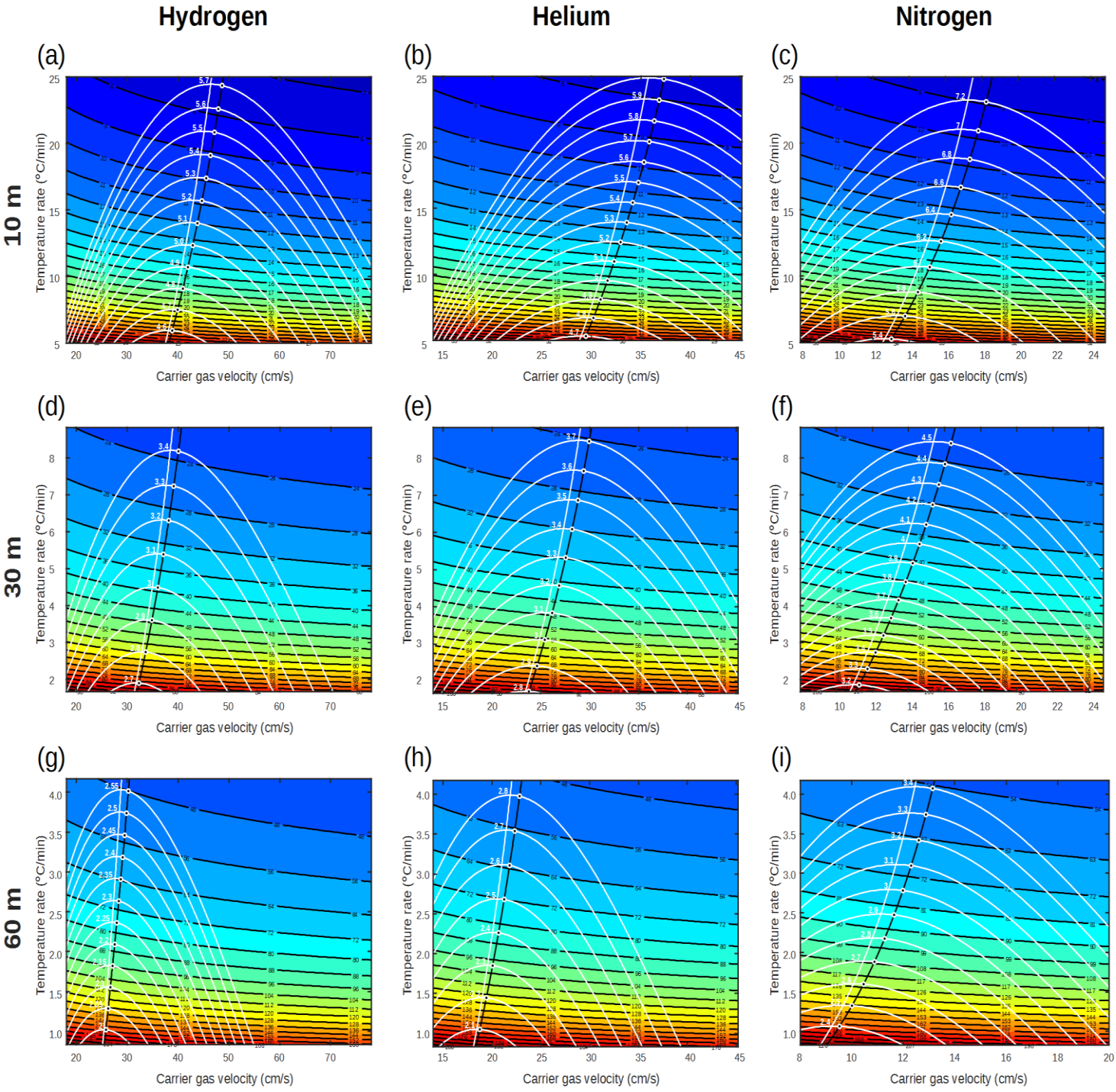

Figure 16: optimal conditions plots for the temperature-programmed pilot experiments

Unlike carrier gas velocity, temperature rate were varied depend on the column length. Higher temperature rate set for short column length while lower for long column length. Since longer column is greatly affected by the temperature rate than short column length does (fig.16).

5.3.1 The van Deemter in programmed temperature in the pilot study

N and inverse of peak width are used to measure the efficiency of column performance in isothermal and temperature programmed respectively. In isothermal condition, Van Deemter equation is expressed by plate height as function of carrier gas velocity whereas in programmed temperature, base peak width (wb) is replaced H [22]. Thus W_b,RI unit, can be used to model van Deemter equation in temperature programmed GC. The models were validated by measuring peak width of against to the average carrier gas velocity and temperature rate. The peak width of the alkanes were obtained from experimental condition of Table 3. The mean and sum of model of van Deemter in peak width of the alkanes was good fit with van Deemter equation for all column dimension (fig.18, left). As shown in the table 9, the contribution of eddy diffusion is observed even if the column was capillary column; the A term might be from extra coulmn effect. For the model, the influnce of experimental condition clearly be relalised.The over all R-squared (R^2) and lower RMSE were calculated for 60m column length and the reverse is true when we used 10m column length. On the other hand, when nitrogen was in the column, over all R-squared (R^2) and lower RMSE value of the model were found. This means, for higher value of R^2 and lower value of RMSE the best fit of the data with model. Of course all the average peak width model of the compound, in all column dimension and carrier gas types, were good fit with van Deemter model equation.

Table 9. A term in three carrier gases within five level of temperature rates and 10, 30 & 60m

10m			30m			60m		
N2	H2	He	N2	H2	He	N2	H2	He
1.695	1.373	0.934	0.708	0.556	-0.141	0.332	0.131	-0.350
1.320	1.273	0.731	0.629	0.498	-0.296	0.077	0.172	-0.419
0.945	1.172	0.527	0.551	0.441	-0.452	-0.175	0.212	-0.487
0.570	1.071	0.324	0.473	0.383	-0.607	-0.428	0.252	-0.556
0.195	0.972	0.120	0.372	0.308	-0.810	-0.683	0.293	-0.625

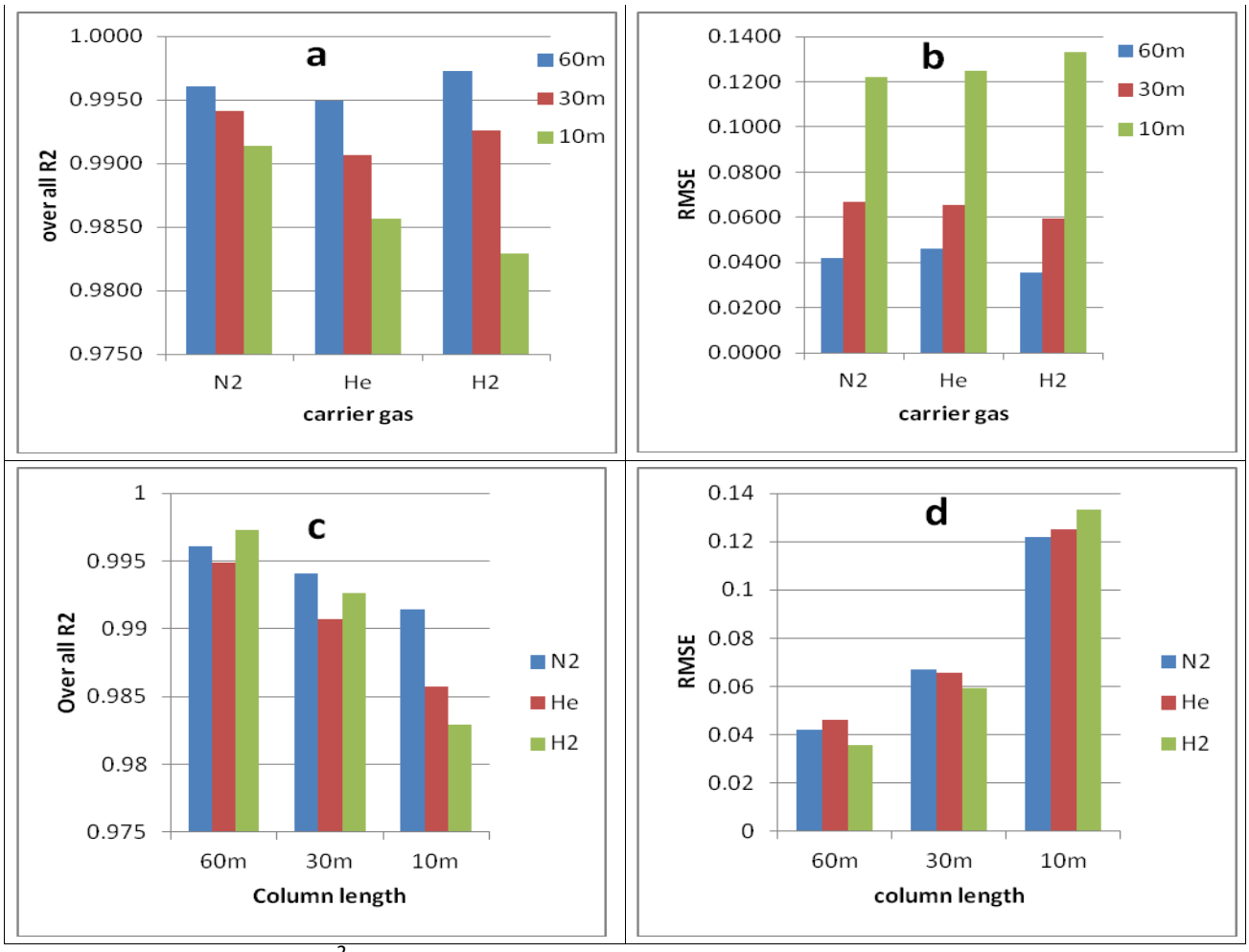

Figure 17 : Overall mean R^2 and RMSE of models obtained from peak width (in five level of *temperature rate)*

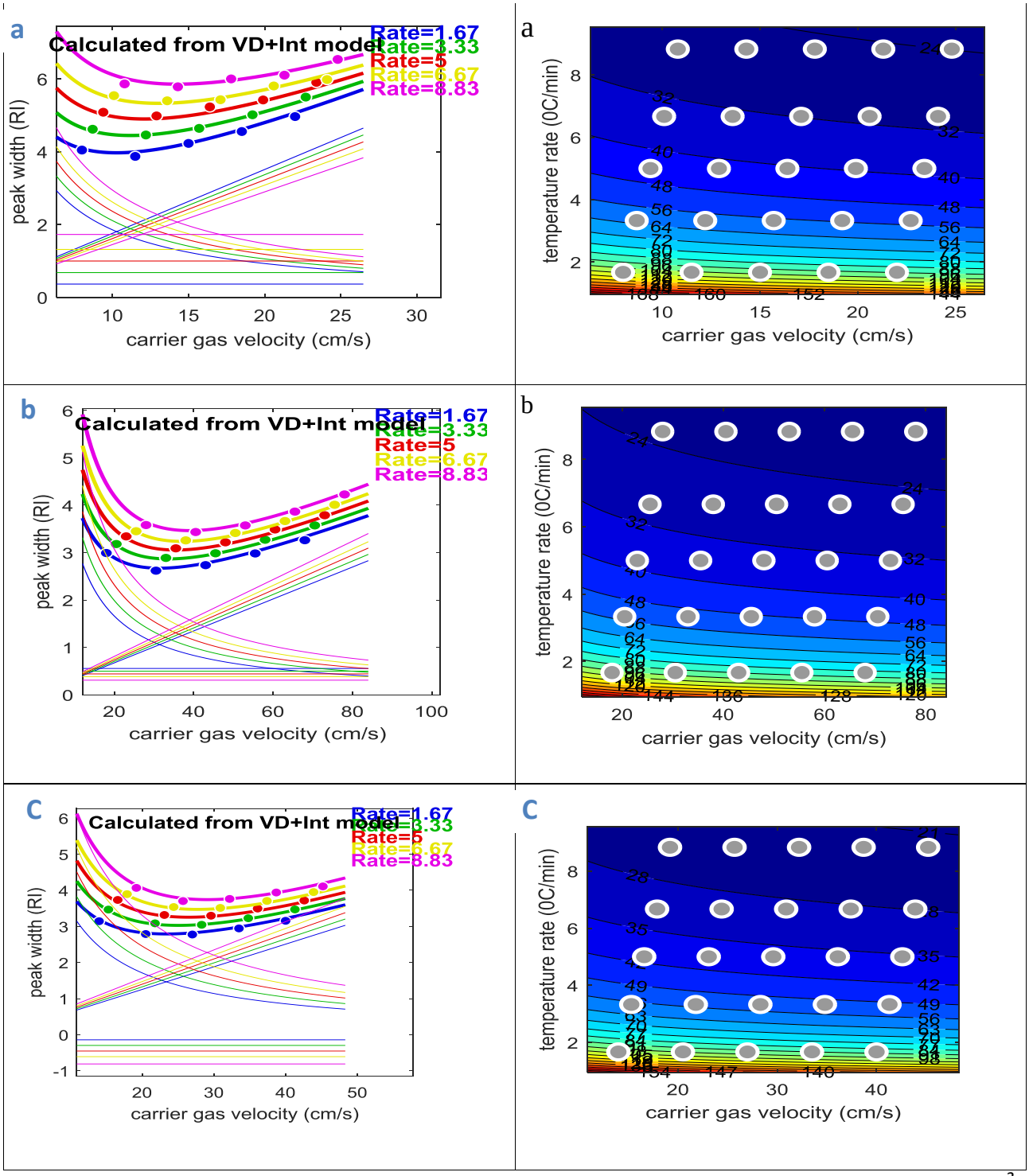

*Figure 18: VD (Peak width model) calculated from VD+I (left) (a) N_2 peak width response R^2= 0.9941 and RMSE = 0.0669 (b) H_2 (peak width response R^2=0.9926 and RMSE = 0.0593 (C) He (peak width response R^2= 0.9926 and RMSE = 0.0655). retention time response surface calculated from Log+I (right) (a) N_2 (time response R^2 = 0.9995 and RMSE = 0.7761) (b) H_2 (time response R^2 = 0.9995 and RMSE = 0.6652) (C) He (time response R^2 = 0.9997 and RMSE = 0.5101) obtained from (**3**0m×250μm×0.25μm, programmed temperature condition).*

5.5 Response surface equations for peak width (wb)

In normal van Deemter equation, efficiency of column is affected by average carrier gas velocity for isothermal condition. As discussed in section 5.3.1, the peak width, in retention index unit, was used to the express the van Deemter equation with taking into account the temperature rate in programmed temperature condition. In this condition, the efficiency of the column performance was greatly affected by both the carrier gas velocity and temperature rate. Response surface equation is applied to describe van Deemter model of peak width as a function of carrier gas velocity, temperature rate and the interaction between the two variables. All interaction effects may or may not important to the model. Models were obtained from different column length (same stationary phase and same dimension) in helium, hydrogen and nitrogen carrier gas. The signifficance terms are adapted from the work reported in [22] and presented as equation 37**.** Where they worked, RMSE after excluding different terms one at a time following backward elimination procedure was used to decide on the significance of each term. A model with low RMSE and low number of terms is preferred. A backward elimination procedure was followed since evaluating all possible combinations of the terms is practically difficult because of many possible combinations and many experiments to be evaluated.

5.6 Models of retention time

Like the efficiency of column performance, analysis time is affected by both temperature rate and carrier gas velocity. As observed from fig. 18 (right), retention time model was calculated by the last eluted compound that has been anaysed in three column lengths (10m, 30m and 60m) for programmed temperature column condition with five level of temperature rate. The model is calculated using equation 40.

Increasing either temperature rate or carrier gas velocity or else both, the time taking to conduct the analysis required to short the analysis time. For both variables (x), retention time decreased with power function of $y = ax^b$; when they plotted againist to retention time (fig.19). As illustrated in the graph of power function, large negative power are being observed in the plot of carrier gas velocity versuse retention time while lower negative power being reported for temperature rate. Mathematically speaking for the above power function, the more large negative power, the less contribution to reduce the time; and on the contrary, the more low negative power, the higher effect to decrease the retention time. This brings to an end, temperature rate is more important than the carrier gas velocity to have short analysis time.

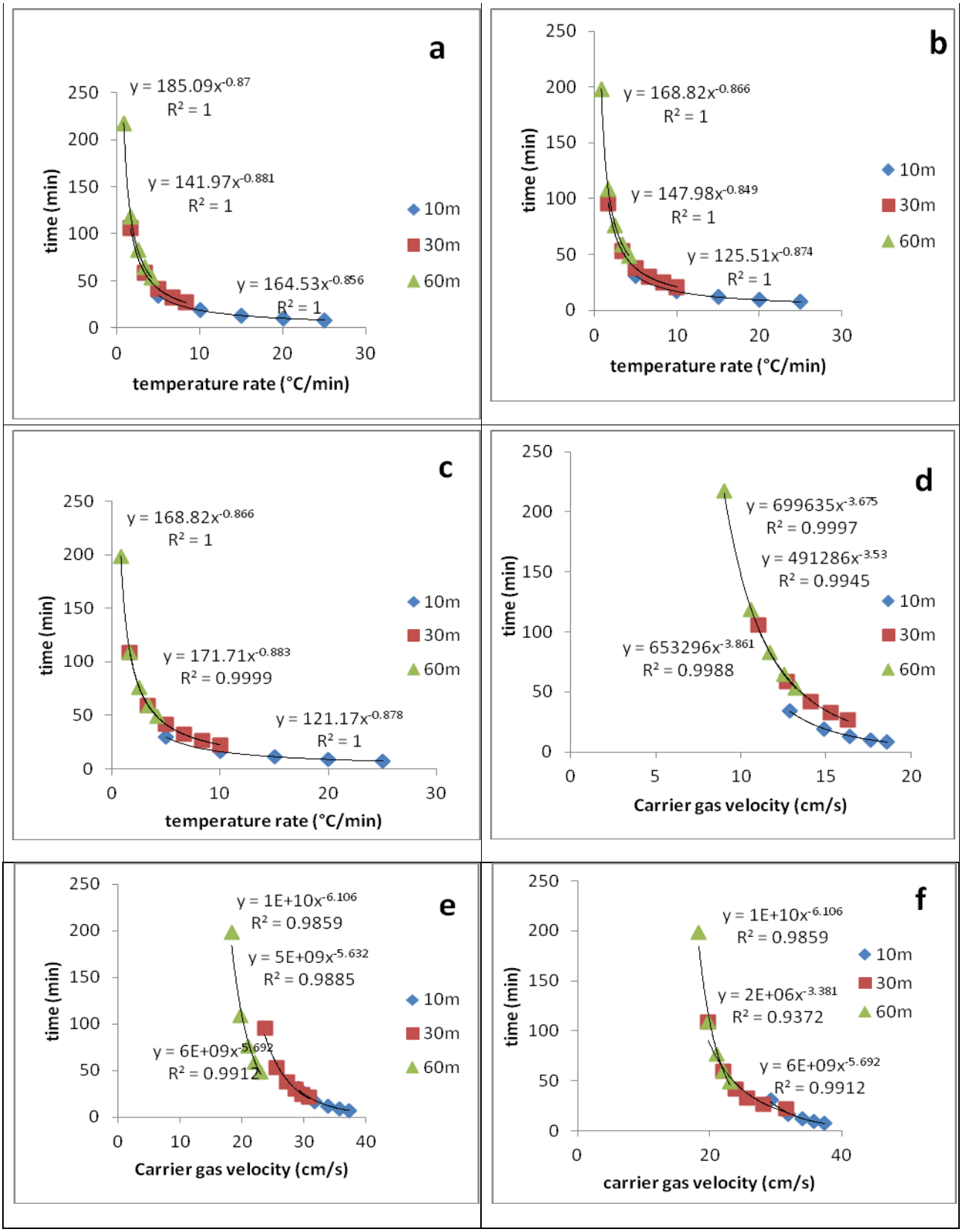

Figure 19: effect of temperature rate and carrier gas velocity on analysis time in three column lengths for carrier gases of (a) N2 (b) He (c) H_2 and (d) N_2 (e) He (f) H_2 (d) respectively in five level of temperature rate.

From the pilot study, we come to the conclusion to main experiment as figured in fig.20. The figure below shows an example of how the carrier gas velocities were standardized as described in the methods section. For temperature programmed conditions the temperature rates and the ratelengths were increased by the factor 6/5. The carrier gas velocities were thereafter set; so that the models predicted similar values at the lowest and highest carrier gas velocities at the lowest temperature rate (bottom left and right corners of the plots), and at u_{opt} at the highest temperature rate. For isothermal conditions the carrier gas velocities were set; so that the predicted plate height at lowest and highest carrier gas velocities were around 0.4. Both for temperature-programmed and isothermal conditions, this required extrapolation of the models. As explained in the experimental section, there were also cases where the predicted range of carrier gas velocities could not be set due to other restrictions.

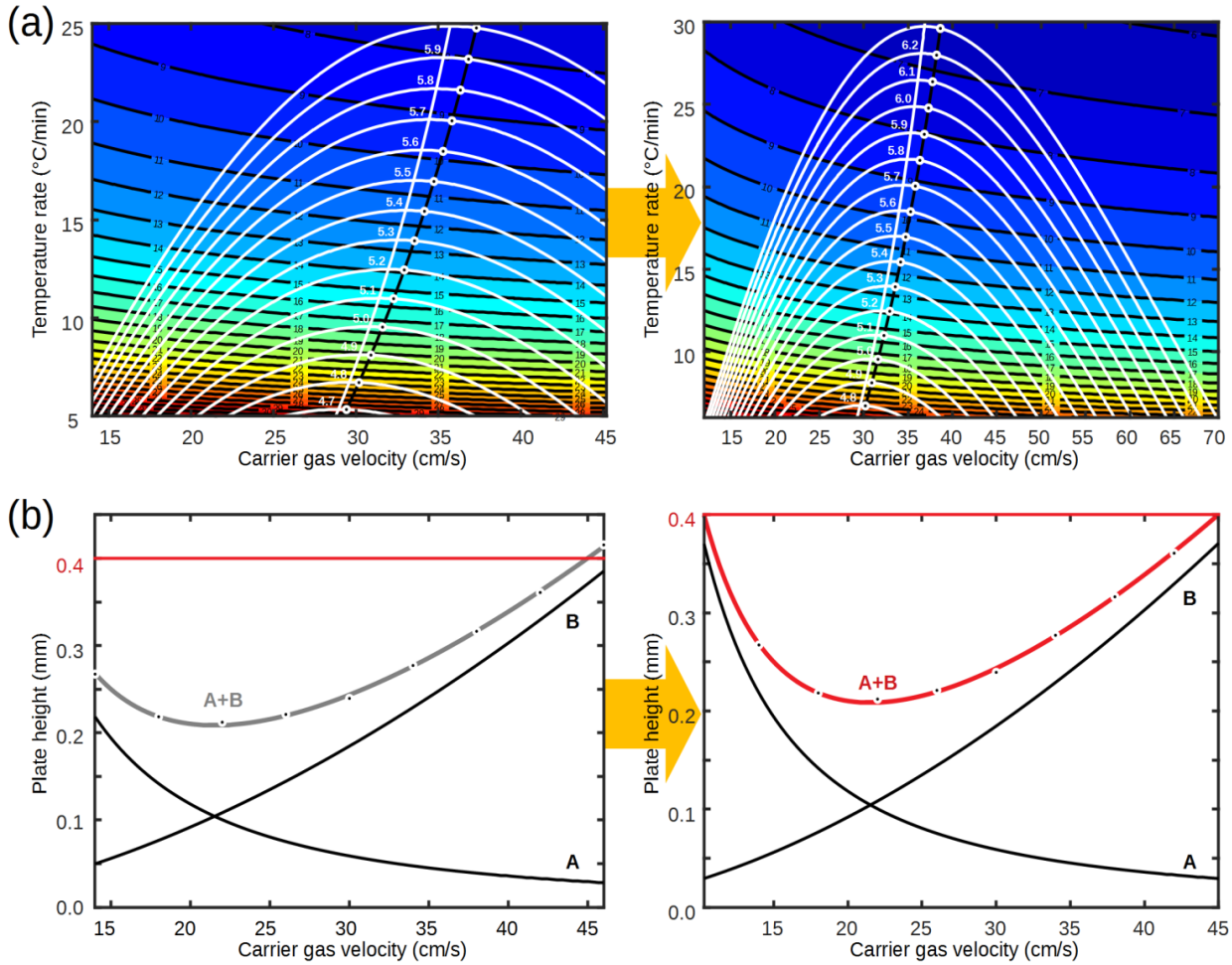

Figure 20: Standardization of the limits for carrier gas velocities shown for (a) Helium at temperature-programmed conditions and column length 10 m, and (b) Helium isothermal conditions and column length 60 m. The plots to the left shows the model within the experimental conditions, while the plots to the right show the models extrapolated to the limits chosen for the new experiments.

5.7 Programmed temperature Gas chromatography

5.7.1 Temperature rate and column length with carrier gases

Alkanes and FAMEs were analyzed by temperature programmed Gas chromatography. The experimental condition were separation column$_4$ in Table **1** and with chromatographic condition of Table 5. When translated the method in between different column lengths, the temperature rates (variable condition) were multiplied with the inverse of the fraction the different column lengths (equation 43) to get approximately the same elution temperatures. This means that the product of the temperature rate and the column length kept constant. The product of the temperature rate and the column length will be referred to as the ratelength and given as m°C/min.

$$\boldsymbol{r_{new} = \frac{L_{old}}{L_{new}}\, r_{old}} \qquad (43)$$

Where r_{new} , r_{old}, L_{new}, L_{old} are the new and old temperature rate and column length respectively.

From this conducted experiment, good enough data is generated and provided in Appendix A, table 18 and Appendix A, fig.37,38 and 39. The inverse of efficiency, peak width model, retention time model and combination of the two models, efficiency/time were calculated, for all column lengths in three of carrier gases. The data from the model used to study the effect of carrier gas velocity and temperature rate in the efficiency and analysis time of GC. The data is also used to explore the appropriate and optimum temperature rate for acceptable efficiency and retention time for each column lengths. The relationship between temperature rate and efficiency in PPC is presented in fig. 21 , $y = ae^{x}$. PPC is the inverse of peak width and calculated using equation 36.

For the same carrier gas and different column lengths, the effect of temperature rate was evaluated. As shown in the relation fig. 21, the efficiency in long column length is greatly affected by the temperature rate than in the short column length did. If the temperature rate of the short column length is extrapolated to long column length, the value of PPC would be predicted to near to zero and the resolution become too poor. Since PPC is directly proportional to the resolution of the chromatogram (equation 34). Fig. 21 tell us the nature of carrier gas can affect the applied temperature rate. For all mobile phase, as the temperature rate increased, the efficiency of separation column decreased exponentially. The efficiency recorded of hydrogen > helium > nitrogen. When the ramp rate increases, the oven temperature also increase; the rising of temperature caused to change the properties of the carrier gases. Unlike the liquid, when gases are exposed to high temperature, the viscosity of gas increased. The viscosity is determined the inlet pressure required for a given gas velocity. High inlet

pressures strongly compress the gas in the column inlet. The compressibility and tolerance of nitrogen to speed for high inlet pressure is the lowest. The diffusivity that provides a measurement for the diffusion speed of a solute vapour in a given gas of the carrier gas is also affected by temperature rate; the higher ramp rate, the lower diffusivities. Helium and hydrogen have with similar diffusivities while that of nitrogen is the lowest [53]. The effect of temperature rate at carrier gases to behave the above changing properties resulted in decreasing the efficiency of the separation column. The range of the useful temperature rates is depending on the diversity of method parameters (column dimensions, carrier gas type, stationary phase type, film thickness, etc.) and thermodynamic factors.

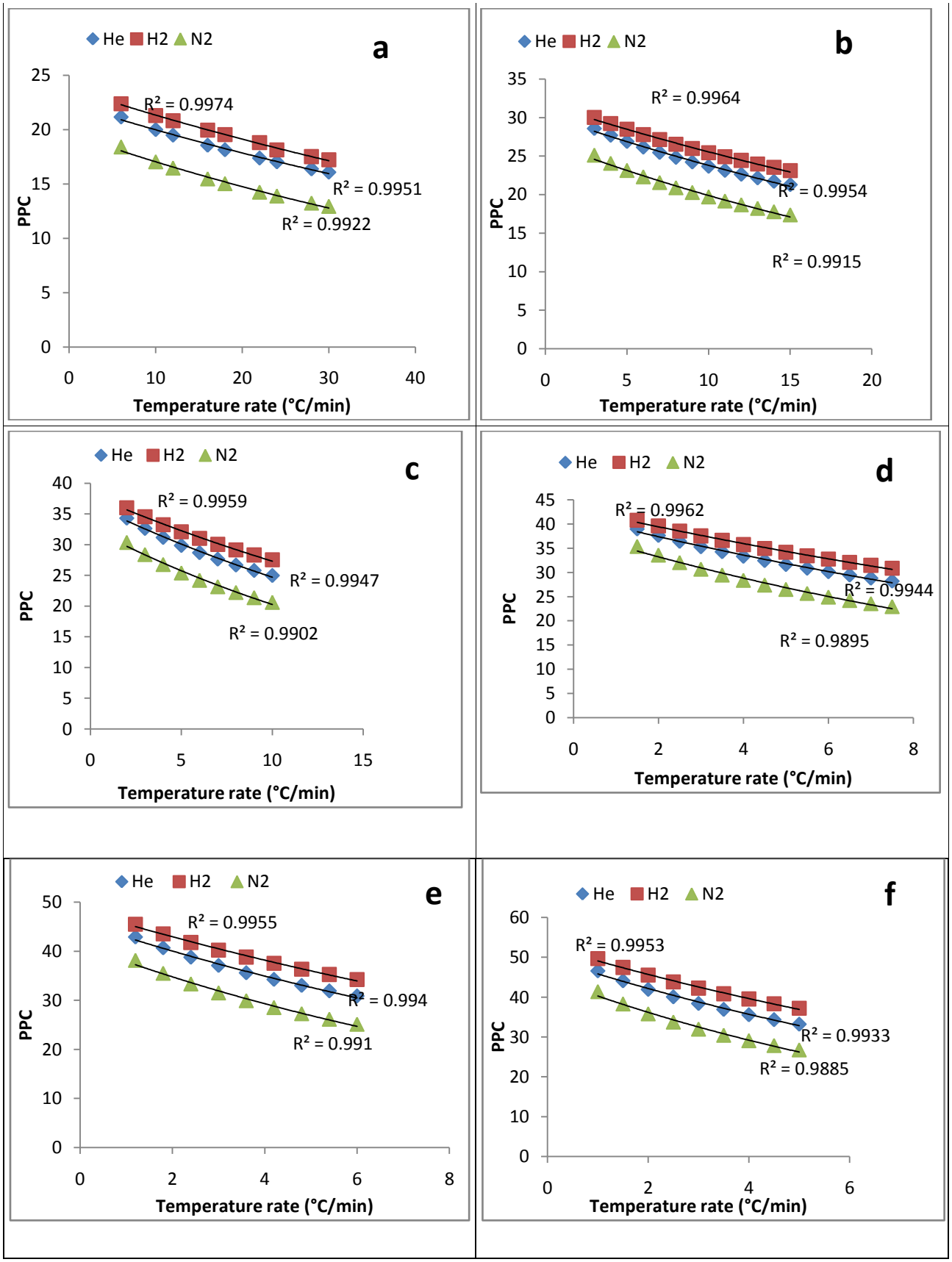

Figure 21: Effect of temperature rate on efficiency column for carrier gases of He, H_2, N_2 in column length (a) 10m (b) 20m (c) 30m (d) 40m (e) 50m and (f) 60m

5.7.2 Optimum temperature rate in programmed temperature GC

Optimum heating rate, in a temperature-programmed GC, is the point where adequate quality of separation and speed of analysis is attained. Giddings [16] had come with the concept of optimum heating rate of the temperature per void time, $t_{M,}$ and empirical formula for evaluation of it. On the issue of ramp rate previously investigated by several scholars to addressee the problem. L. M. Blumberg and M. S. Klee suggested a default optimum temperature rate value of 10°C per void time for all programmed temperature capillary GC [15]. It is claimed that an optimal heating rate that will maximise the ratio of peak capacity to the time it takes to elute each peak, and it is typically used as a "rule of thumb" to set the heating rate in GC. One problem with the advice is to define the dead time. The viscosity of the carrier gas will increase with temperature.

There are typically two pressure modes used in temperature-programmed GC, the constant pressure mode, and the constant flow mode, where the pressure is gradually increased with the temperature to keep a constant volumetric flow of gas through the column. In both cases, the carrier gas velocity will vary with the temperature, it is gradually decreasing with increasing temperature in constant pressure mode and it is gradually increasing with constant flow mode. Another problem with the advice is that it does not require the carrier gas velocity to be optimal (i.e. it can be the dead time of any velocity).

To evaluate the advice and compare with the results from this work optimal heating rates were calculated by the following method. As described in the regression curves (fig.40), When the ratelength is going form 60 m°C/min to further the models become very close and start to overlap. This is the indication where the optimum ratelength is going to be approached and attained. And therefore at 180 m°C/min ratelength is temperature rates were chosen to determine void time for all carrier gas and column length. The optimal carrier gas velocity (u_{topt}) at the ratelength of 180 m°C/min was used as carrier gas velocity. This is the carrier gas velocity at 60°C and is a low estimate of the carrier gas velocity. The corresponding flow in mL/min at this velocity was thereafter calculated. From this flow, the carrier gas velocities at the elution temperature of C_{24} (high estimate) and halfway between 60 and the elution temperature of C_{24} (middle estimate, temperature at half time of equation 26) was calculated. At the low estimates (60°C) the analytes are trapped in the stationary phase, so no chromatography is taking place. At the elution temperature, the temperature calculated by equation 26, of C_{24} (the high estimate), the last compound elutes, so no chromatography is taking place. The low and the high estimates therefore represent boundaries for which carrier gas velocities that can be present, and the middle estimates represent velocities that can be typical (table 19). From the column lengths and the estimates of the carrier gas velocities, the dead times were calculated using (equation 3) and the corresponding proposed optimal heating rates were

calculated (10°C/t_M). The optimal heating rates was thereafter converted to ratelengths, and the relationship between the proposed optimal ratelengths and the column lengths are plotted in the figure below. The decreasing trend for each carrier gas is because u_{topt} decreases with carrier gas.

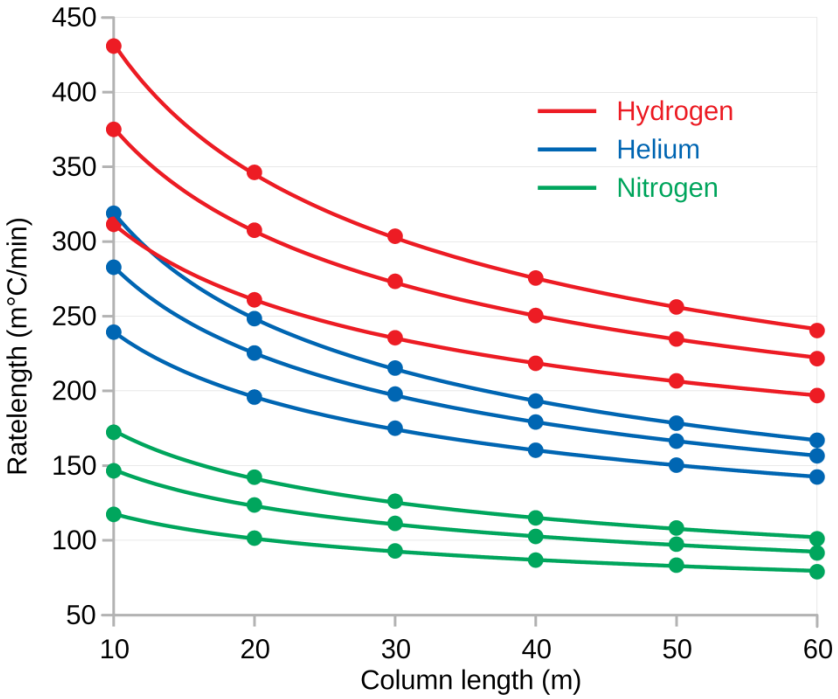

Figure 22: Ratelength vs. column length for calculated optimal heating rates using the 10°C per void time criterion. utopt at a ratelength of 180 was used as initial carrier gas velocity (low estimate). The three curves for each gas represent velocities at the start (low estimate) at the end (elution temperature of C24, high estimate), and at the middle of the chromatogram (middle estimate). Regression curves are fitted power functions.

The advice of 10°C per void time is supposed to give optimal rates for a given column, i.e. the length of the column is constrained. The relevance of the advice can be compared to the data from this work by calculating PPC/t_{topt} for the five rates for each column (PPC of the proposed temperature is calculated from regression curve of temperature rate and PPC in each column length). Figure 23 shows plots of PPC/t_{topt} against ratelength for different column lengths and the three different carrier gases. It can be seen that all the relationship is increasing in all cases and that no maximum in PPC/t_{topt} is found within the conditions tested. The proposed optimal ratelengths are shown in the same plot. Although the development in PPC/t_{topt} seems similar for all columns and carrier gases, the proposed optima are very different.

The similarity between column length and carrier gases in the development of PPC/t_{topt} becomes more obvious when we look at the percent change in PPC/t_{topt} when ratelengths are raised by 60 m°C/min (fig.24). Then there is almost zero differences

between column lengths and carrier gases. Considering these similarities it is strange that the proposed optimal rates are so different. The conclusion from our data indicates that the proposed optimal heating rates are not optimal.

Another question is if considering PPC / t_{topt} is the best way to optimise the system. It has been claimed previously [22] that one have to put a constraint on either PPC or t_{topt} to find optimal conditions. This can be illustrated by considering the relationship between PPC/t_{topt} and the column lengths. This is shown in fig. 25. It can be seen that PPC/t_{topt} increases with decreasing column length. The regression curves are power functions with negative exponents, which will approach infinity as the column length approaches zero. Thus, the logical consequence is that the column length should be zero to maximise PPC/t_{topt}, which of course will not give any separation at all.

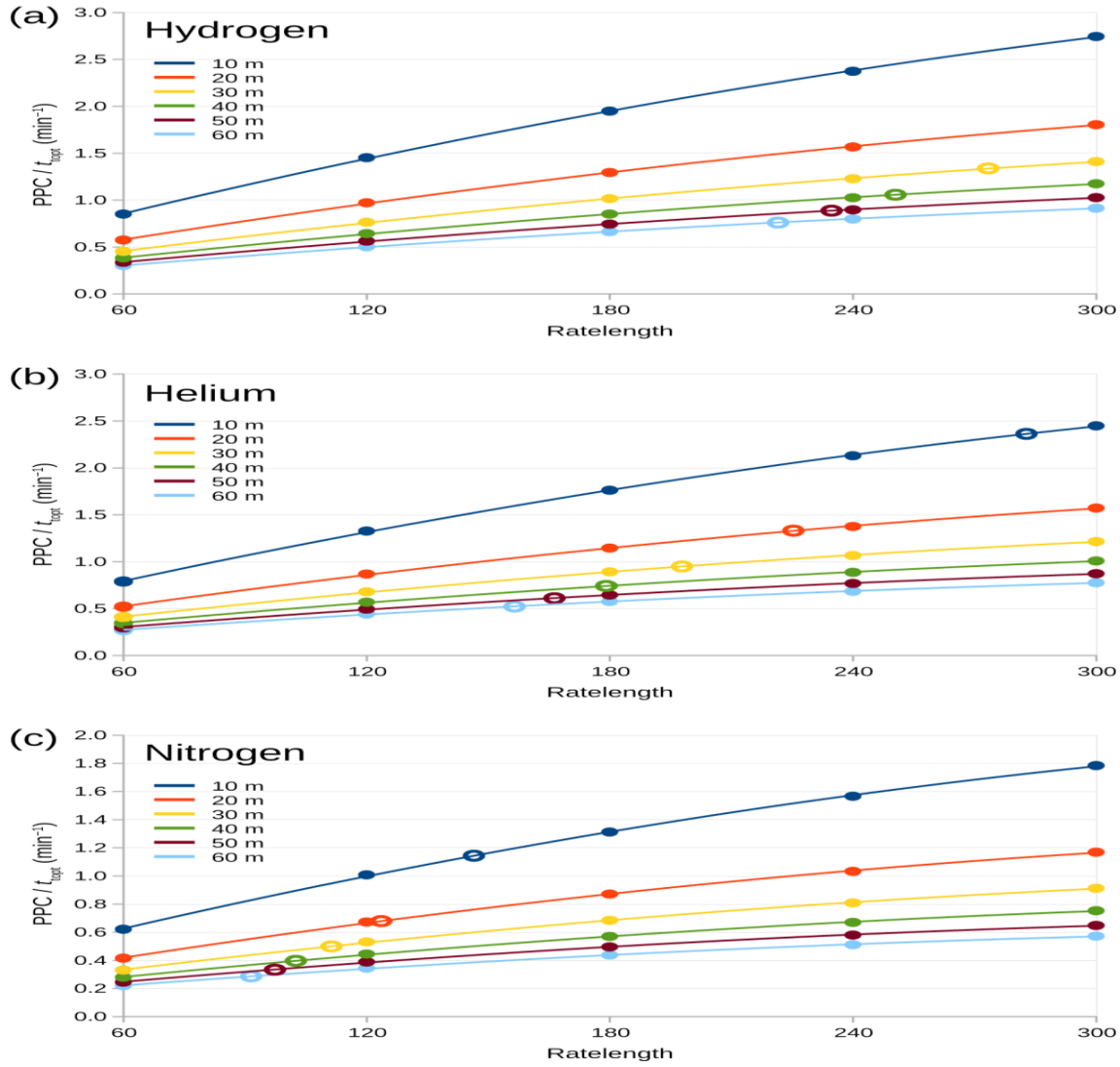

Figure 23: Plots of PPC / t_{topt} against ratelength for different column lengths and the three different carrier gases, hydrogen (a), helium (b) and nitrogen (c). Open circles illustrate the proposed optimal ratelengths by the 10°C/min criterion (middle estimate).

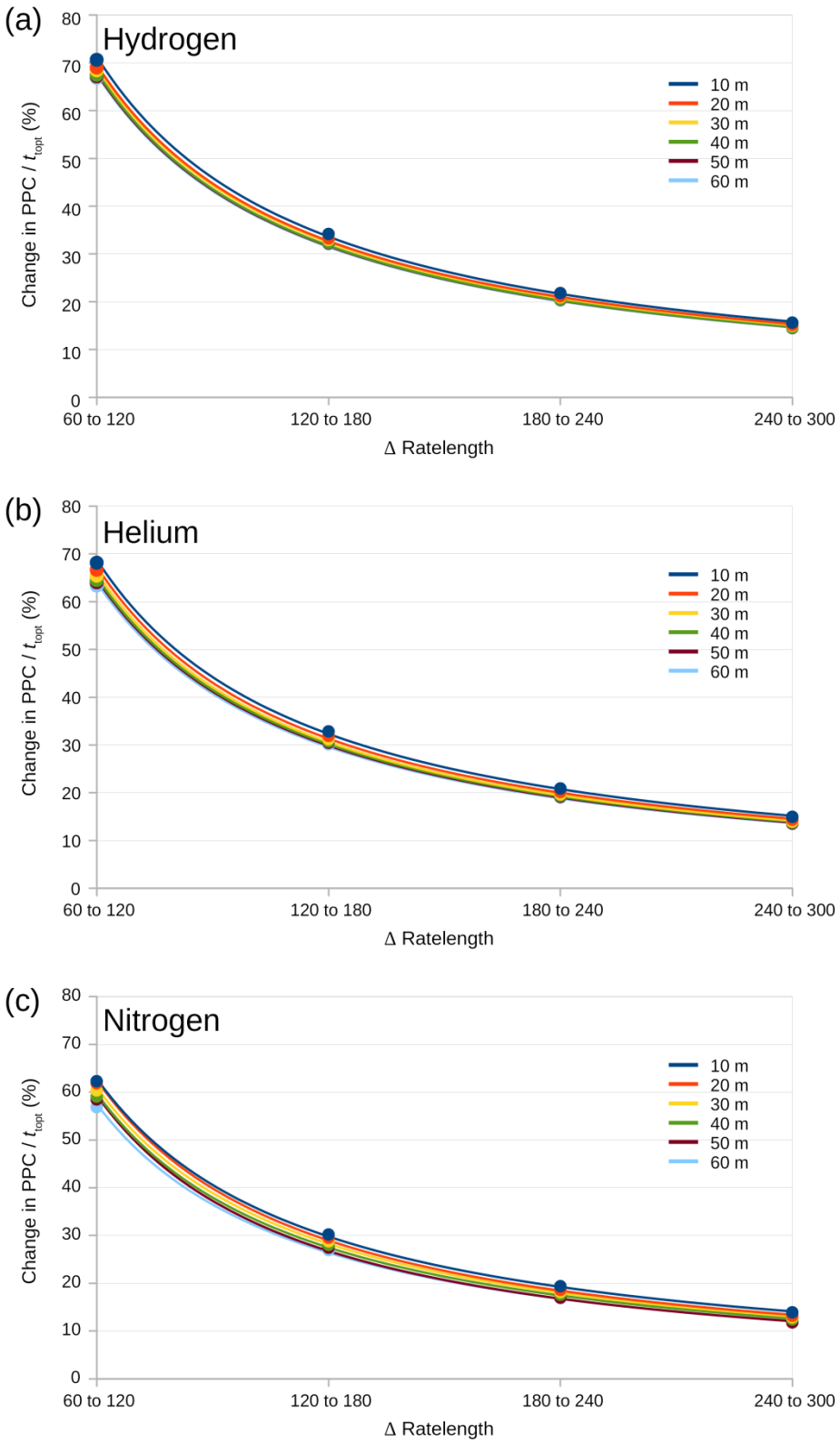

Figure 24: Percent change in PPC/t_{topt} by increasing the ratelength by 60 m°C/min.

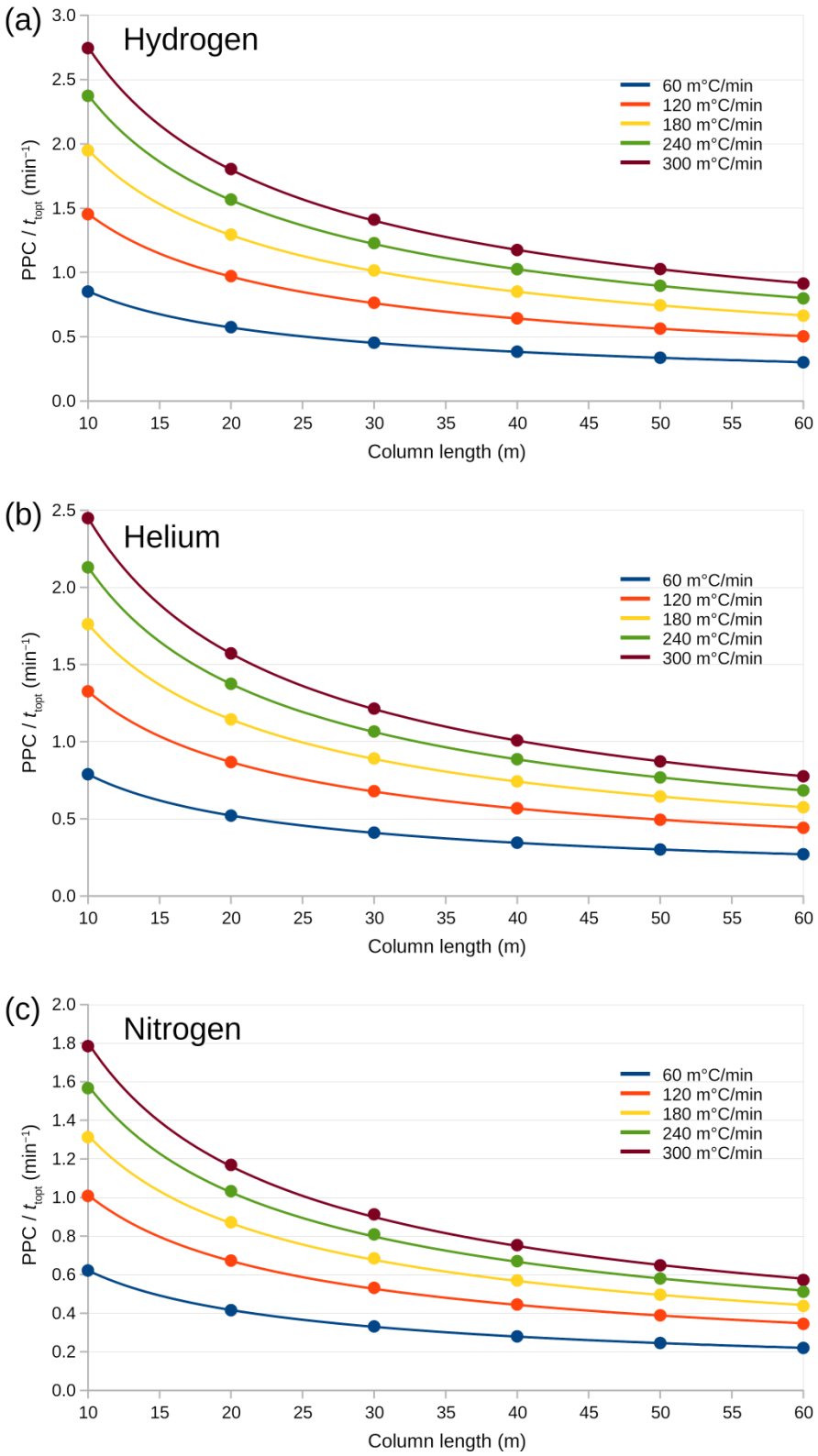

Figure 25: Plots of PPC / t_{topt} against column length for different ratelengths and the three different carrier gases, hydrogen (a), helium (b) and nitrogen (c).

5.7.3 Comparison of column performance in PTGC

Column dimension is as important as the role of nature of stationary phase in the analysis of samples. The selection of column dimension for the analysis of FAMEs and alkane samples therefore should be depend on the performance of the columns, both with regard to efficiency and time of analysis that the analyst wants to attain.

The performance of column 4 for the analysis of FAMEs and alkanes were evaluated and compared. The efficiency, and time at optimal velocity, ratelength, temperature rate, using the three carrier gases, were presented in Appendix A, fig.37,38 and 39. As described in the fig.28, in all three carrier gases, 60m column length is found to be the most efficient columns in the analysis of the sample whereas the 10 m column is less efficient. In fact, when the length of the column increased from 10 m to 60 m the efficiency of the column increased with punishment of long analysis time.

The effect of carrier gas transition in the performances of different columns length is tried to have a look at, within same ratelength. As described in fig.26, the transition of column length in a single carrier gas type, there is no as such big observable change of efficiency and time through the entire. The cause for the existence of bit change, in between the column length, is the column conditions (pressure drop, pressure is increased when column length increases). For having relatively equivalent efficiency is because the mobile phases (carrier gas) do not interact with the stationary phase and as well the solute (alkanes and FAMEs) and the column is composed of same chemistry of the stationary phase, internal diameter and film thickness. Whereas when we replaced one carrier gas by the others, for same column length and ratelength, there will big difference in both efficiency and analysis time (fig.27 c). Helium is chosen as reference which shared properties in between nitrogen and hydrogen. Thus by changing the carrier gas from helium to hydrogen, helium to nitrogen to see the gain or loss in efficiency and/or time are determined. The effect of the three carrier gases in the efficiency of separation column and analysis time, were determined. And therefore, for all employed column lengths, replaced helium by hydrogen, nitrogen by helium the efficiency will be improved by 7%, decreased by 16% respectively. On the contrary, the time required for the analysis, for the above switching on carrier gases, are decreased by 5% and increased by 10%.

In fig.27 c, the efficiency which has been recorded by 60m column length, for nitrogen, is nearly equivalent to the efficiency of 30 m for H2 carrier gas and the analysis time required for hydrogen is reduced by half of the time consumed for nitrogen. Likewise, the efficiency obtained from 60m column length and 50 m is nearly equal for He and H_2 in the column as carrier gas respectively. we can lower the analysis time to the period of 0.82 times the time for helium while switching of hydrogen. Installing 40 m separation column, with carrier gases of hydrogen and helium, we can achieve 1.12 times and 1.05 times more efficiency than the efficiency obtained from 60m column length for nitrogen in the column and the time needed for the analysis is

decreased to 3/4 of the time taken by nitrogen in 60m. The peaks per carbon determined in 50m column length with helium and nitrogen as a carrier gas are 1.01, 1.23 times lower than the efficiency the separation by 40m column length with hydrogen as the mobile phase; therefore we can get a reduced the analysis time equal to 0.9 times of the analysis time required by 50m separation column when Helium and nitrogen are in the column.

Apparently, best efficiency is being obtained from long column length, this is true when carrier gas type is same. And hence, carrier gas type can bring a very significance influence in both the time to conduct the analysis and the performance of the separation column since the properties of carrier gas contributing to increase plate height as provided in van Deemter curve and expanded Golay equation, equ. 18. This shows that to take an opportunity of to have acceptable and better efficiency with low analysis time by switching of carrier gas from nitrogen, long column length, to hydrogen and helium with short column dimension using the appropriate experimental condition. The contribution of carrier gas type for efficiency of in various column length is noticeably observed for longer and medium length but not near to short and exactly short column lengths.

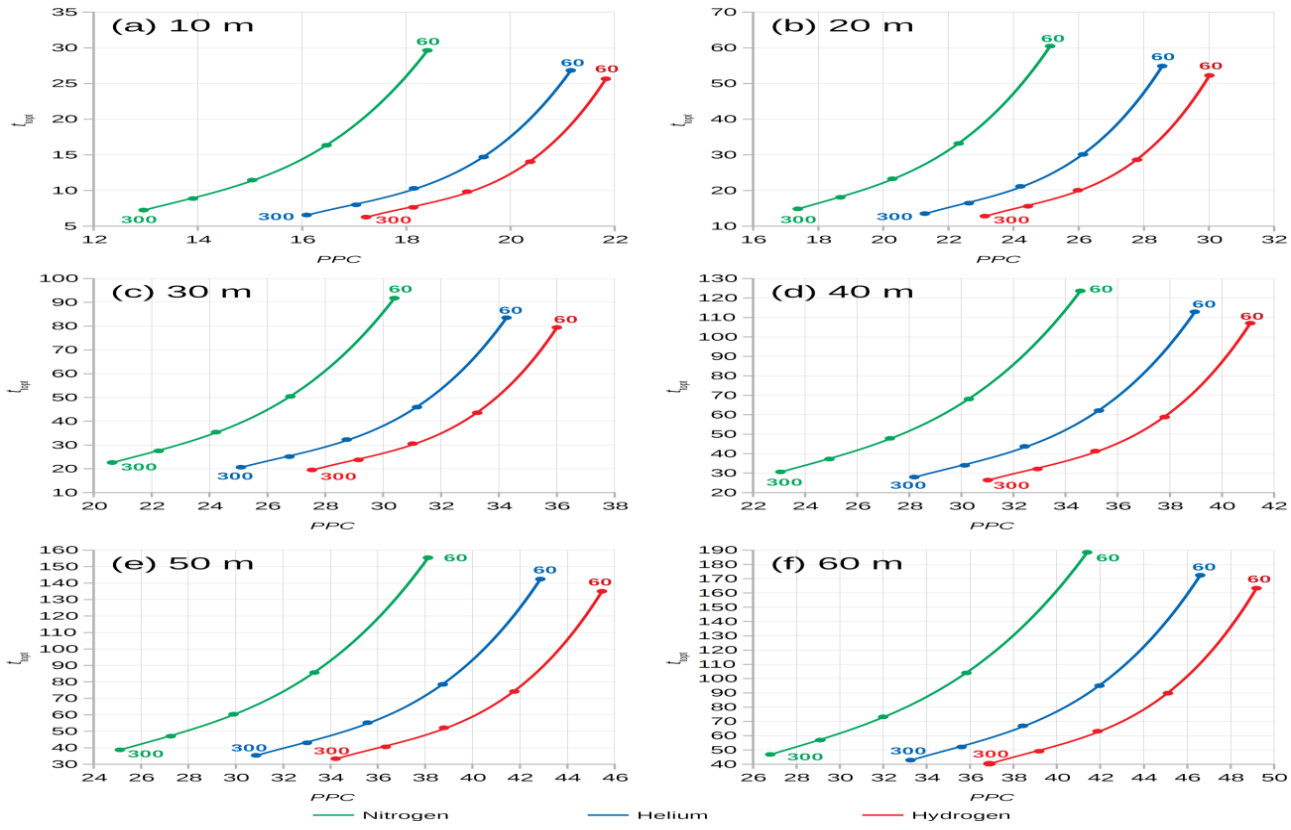

Figure 26: PPC from optimum time for three carrier in the column length a) 10m b) 20m c) 30m d) 40m e) 50m f) 60m. Numbers on the extremes of the curves show ratelength in m°C/min.

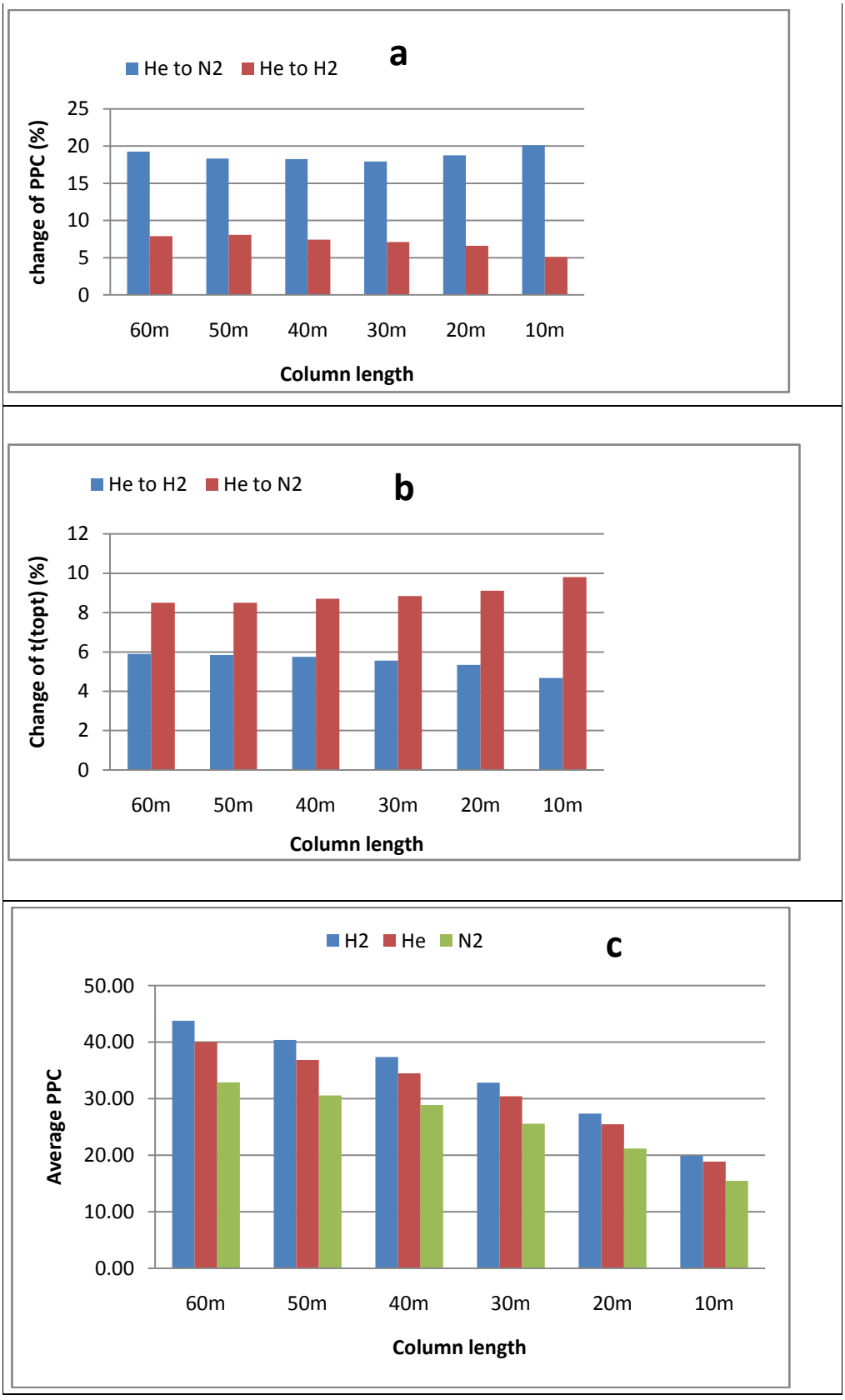

Figure 27: rate of efficiency (a) time (b) when switched one carrier gas over the others. Effect of carrier gas in time-Efficiency (c) in different column lengths (007-5 Quadrex, 10, 20, 30, 40, 50, 60m 250$\mu m \times 0.25 \mu m$).

5.7.3.1 Transition effect

As clearly observed from the fig.28, for same ratelength and for all carrier gases, when we shift from 10 m to 20 m, 20 m to 30 m, 30 m to 40 m, 40 m to 50 m and 50 m to 60 m the average efficiency will be increased.

The plot below show the effect of adding 10 m column length and keeping the ratelength, which is a type of column transitions. Fig.29 (a) show that the increase in t_{topt} goes clearly down as the ratelength increase, which is as expected. Fig.29 (b) show that the absolute increase in PPC goes clearly down with the column length, *i.e.* increasing from 10 to 20 m has a much more positive effect on the column capacity than increasing the length from 50 to 60 m. For the t_{topt} there is close to no effect of the column length, but there is a small positive trend within each ratelength and minor differences between the carrier gases (plot a).

Things get more interesting when we look at the percent changes, $\Delta d_{\%}$:

$$\Delta d_{\%} = 100\% \frac{d_{+10\mathrm{m}} - d}{d}$$

where d is t_{topt} or PPC of the short column, and d_{+10m} is the corresponding values of the 10 m longer columns. The percent increase in t_{topt} (fig.29(c)) follows the percent increase in column length, but is marginally higher because u_{topt} decreases with column length. The values slightly above 100% is from the increase from 10 to 20 m, which is a 100% increase. The values slightly above 50% is from the increase from 20 to 30 m, which is a 50% increase. There is no significant separation between values for different carrier gases. The percent increase in *PPC* (plot d) follows a similar trend, but with some separation between the carrier gases and a marginal trend towards lower values with increased ratelength. However, the percent increase in *PPC* is much lower than that the percent increase in time, with the largest values around 35%, that correspond to the 100% increase in column length.

If we take the fractions of the numbers shown in plots, fig.29(c) and (d) we get the numbers shown in plot (e). The majority of the values are between 0.3 and 0.4, with an average of 0.36. That means that we can make a quite general conclusion: irrespective of the carrier gas, the temperature rate and the column length, we can expect to get 30-40% increased peak capacity compared to the investment in increased time. There are some trend that are difficult to see in the plot. All carrier gases show a small reduction with ratelength. The average fraction at 300 m°C/min is approximately 90% of the average at 60 m°C/min. Increase in t_{topt} and *PPC* by adding 10 m column length and keeping the ratelength. (a) absolute increase in t_{topt}, (b) absolute increase in absolute increase in *PPC*, (c) percent increase in t_{topt}, (d) percent increase in *PPC*, (e) fraction of values in (c) and (d). Special attention should be given to the column dimension that shows us to take advantage with acceptable and good enough

efficiency with low analysis time among the column dimension using the experimental condition.

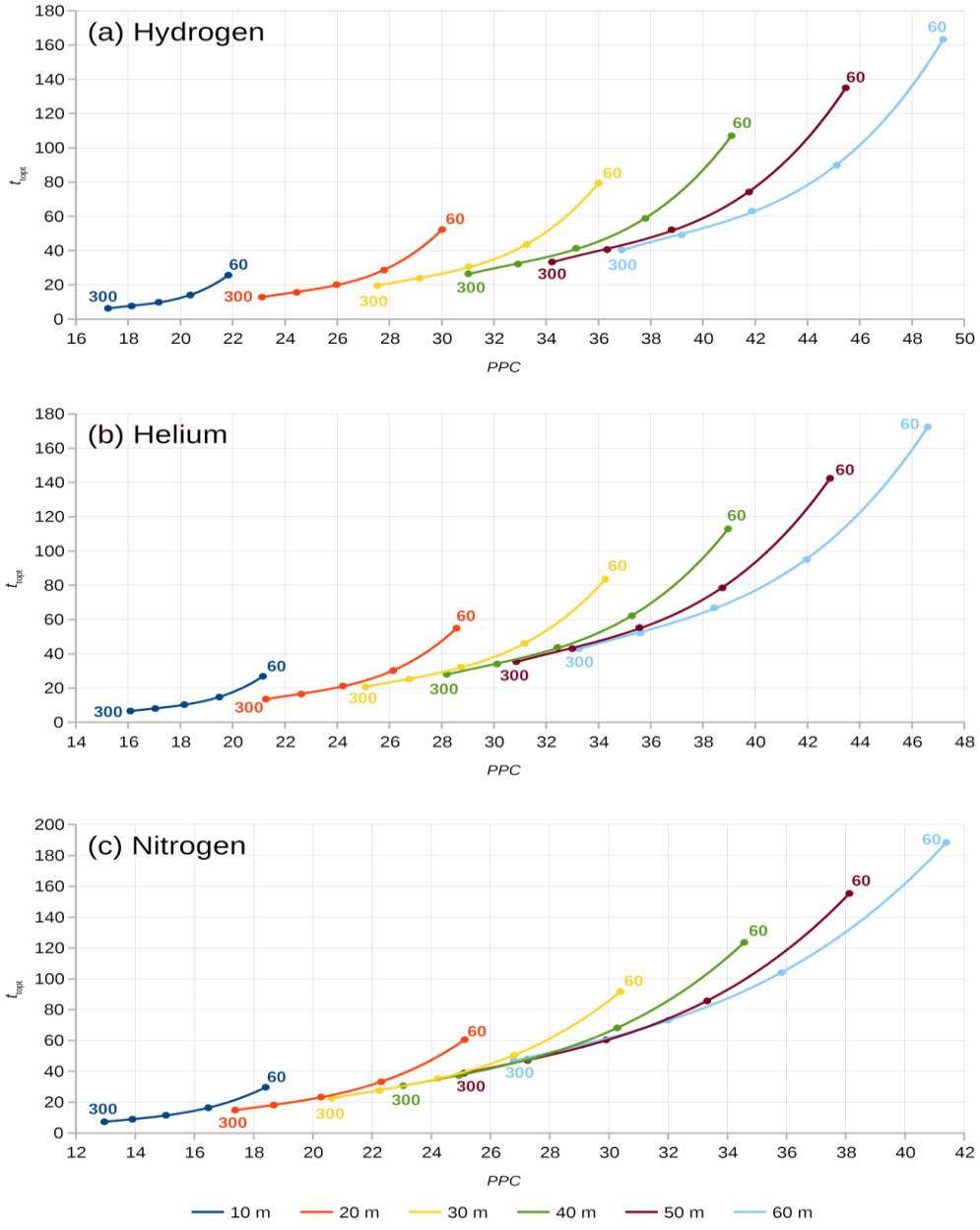

Figure 28: Different column lengths compared for different carrier gases. Numbers on the extremes of the curves show ratelength in m°C/min.

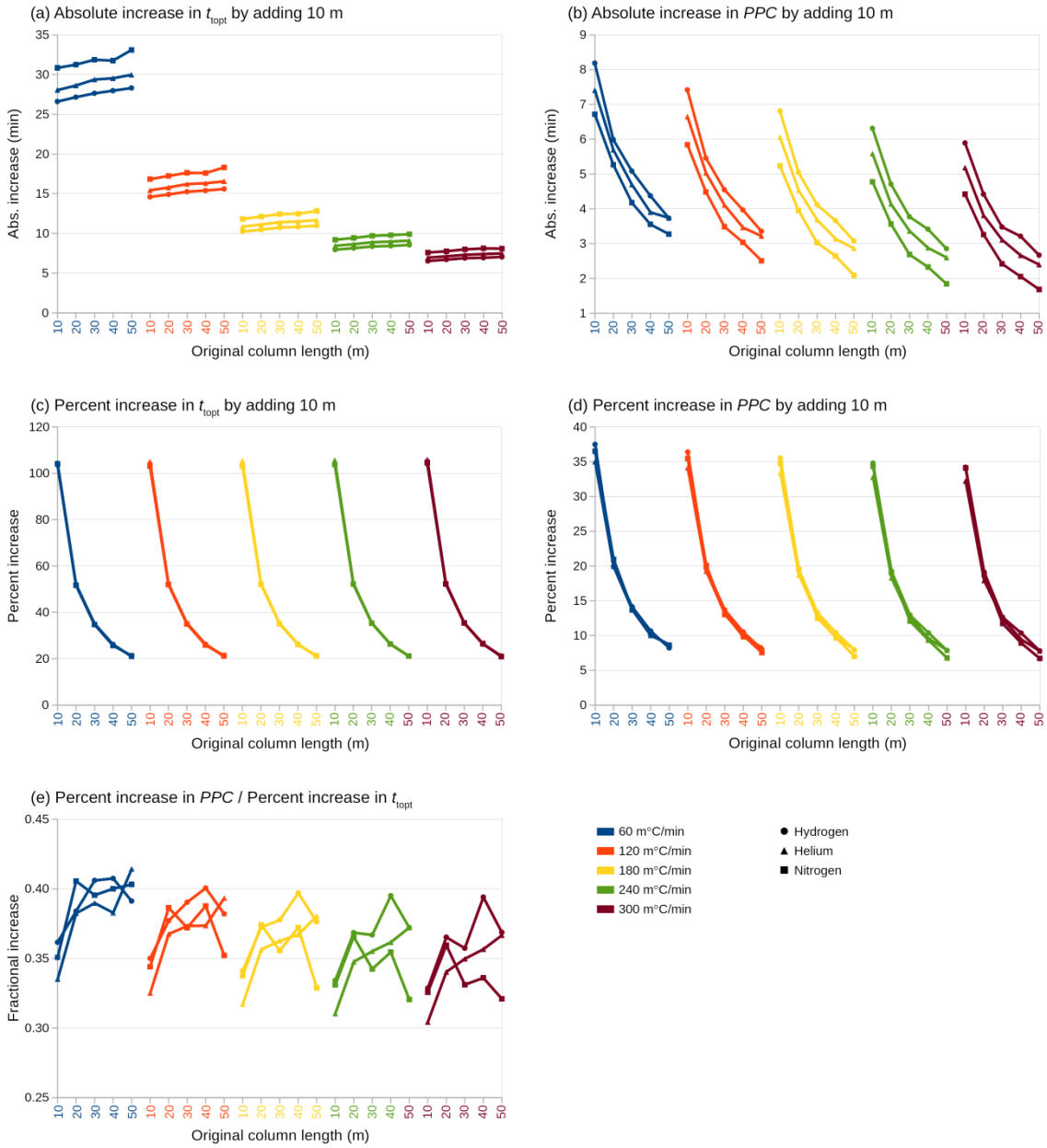

Figure 29: effect of transition (a) absolute increase in t_{topt} (b) absolute increase in PPC (c) percentage increase in t_{topt} (d) percentage increase in PPC (f) ratio of percentage increase in PPC/percentage increase in t_{topt}.

5.8 Dispersion model

Alkane and FAMEs were analyzed by capillary GC. He, N_2 and H_2 as carrier gases with column 4 were employed with the condition of Table 4. For each column dimension and carrier gases dispersion models, plate height, were calculated. The models were investigated how to fit the data to the expected model by taking consideration of the effect of column dimension and carrier gas nature.

5.8.1 Effect of column length

The efficiency of column performance in isothermal gas chromatography is evaluated by the plate height of the column [22].The smaller plate height (H), the better column efficiency. Height equivalent to one theoretical plate, H, had been described, as function of average carrier gas velocity; the compressibility of the carrier gas is being caused not to have a good fit to experimental data for long column length [32]. A challenge when studying plate height in capillary columns is the mobile phase (carrier gas) is compressible, which lead to higher density in the beginning of the column, where the pressure is high, than at the end, where the pressure is low. Because of the difference in density, D_M will be gradually increasing from the injector to the detector. Because of this, Equations 16 to 18 will only fit well to experimental data when the pressure drop is low (short wide bore columns).

In our work, five mathematical models (Golay) for plate height were calculated with different level of pressure drop for all column lengths. These five models were presented in table 10 with overall R-squared and RMSE. Equation 16, 19, 20 and 21 are used to calculate the plate height of the Golay model.

Models of FAMEs and alkane compound were obtained from plate height using the above stated Golay and van Deemter mathematical formula. Over all R-squared (R^2) and root mean square error (RMSE) were used to compare, develop and evaluate the models from the results. As illustrated in fig. 30, when the column length increased, the correlation coefficient of the Golay model (low PD) decreased, for all carrier gases; while root mean square error (RMSE) of the model was increased. Unlike Golay model at low PD, the overall value of R-squared (R^2) and RMSE of the Golay model at high PD inclined up and decreased respectively from short column length to long column length. Whereas in the rest Golay model and van Deemter, the value of overall R-squared (R^2) and RMSE are not influenced substantially by column length particularly Golay in interim PD and van Deemter; in fact the value of overall R-squared (R^2) were high and RMSE with low value. High value of R-squared (R^2) and too low RMSE are recorded in the model of Golay. Low value of R-squared (R^2) and high value of RMSE are obtained in the Golay model at low pressure drop in all column lengths for all carrier gases. Golay model at low pressure drop is good fit for Short column length. Irrespective of column dimension, good fit models of plate height were designed by the Golay model with interim pressure drop and van Deemter too. obviously, van

Deemter model is not essential in our work since the experiments were carried out by capillary column, with no packed material that brings eddy diffusion.

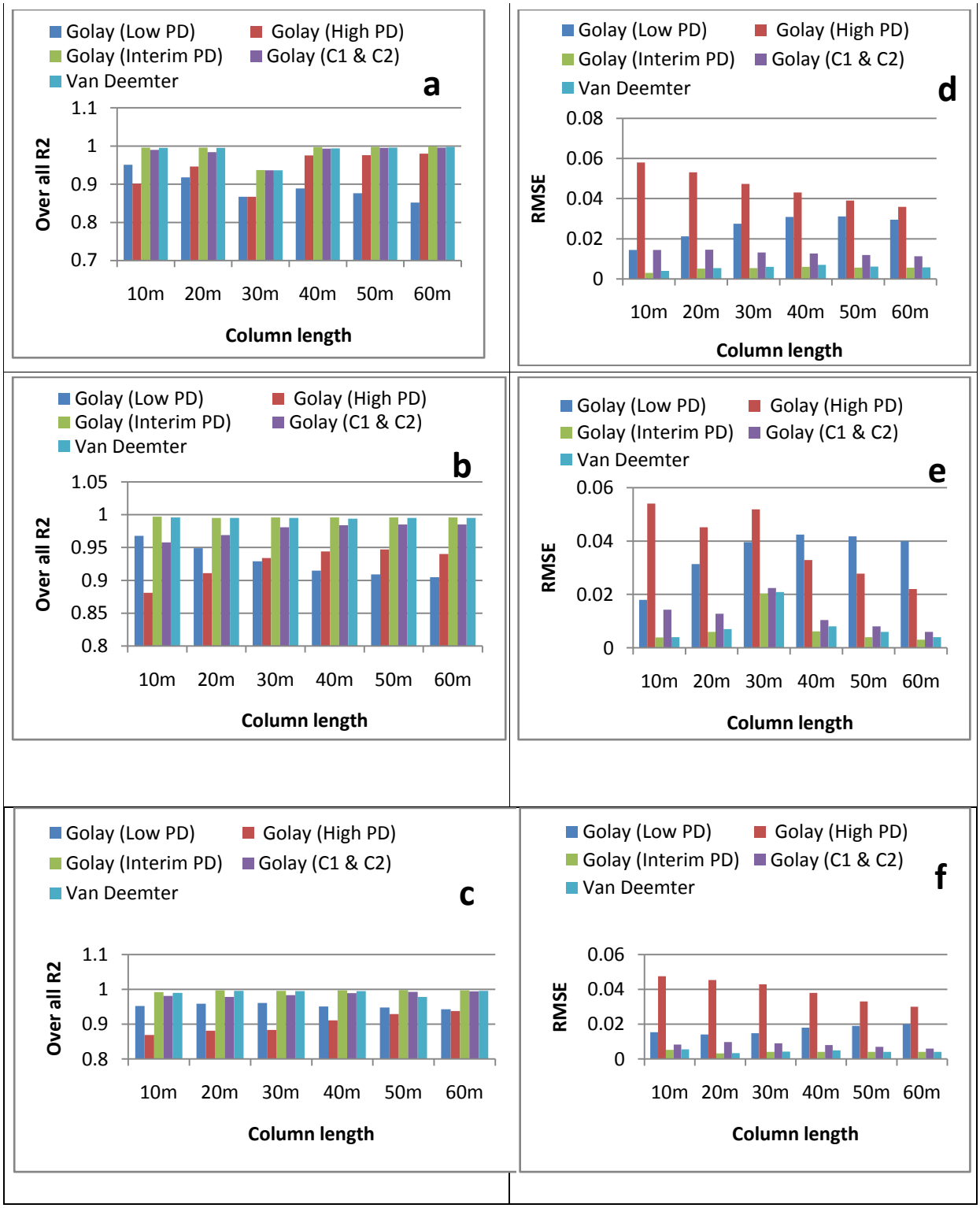

Figure 30: Over all R^2 (left) and RMSE (right) of models obtained with plate height using as (a) Hydrogen (b) Helium (c) Nitrogen and (d) Hydrogen (e) Helium (f) Nitrogen .

5.8.2 Effect of carrier gas

As illustrated in fig.31 and table 10 (A, B), the models of plate height were evaluated by their overall R^2 value for each column length in all carrier gases. The higher over all R^2 close to one, the better the calculated model and the better the data fit with the model. In fact, all dispersion equations are affected by the nature of carrier gas but the first two models, Golay at low pressure and Golay at high pressure are highly practical. Since carrier gas influences a GC separation in two significant ways. First, carrier gas linear velocity determines the speed at which solute molecules move along the column while in the gas phase. The pressure drop across the column required to attain a specific average carrier-gas linear velocity is related to the column length, the diameter, compressibility, diffusivity and the gas viscosity. Longer GC columns or higher gas a viscosity requires a higher pressure drop to yield a given average gas velocity. Still experimental data is best for Golay model with interim pressure and Van Deemter too; however, Van Deemter is not acceptable any more since the installed GC column is capillary column which does not have multiple path effect. Generally speaking, the best fit model is Golay in interim pressure regardless of the nature of carrier gas and the dimension of the column.

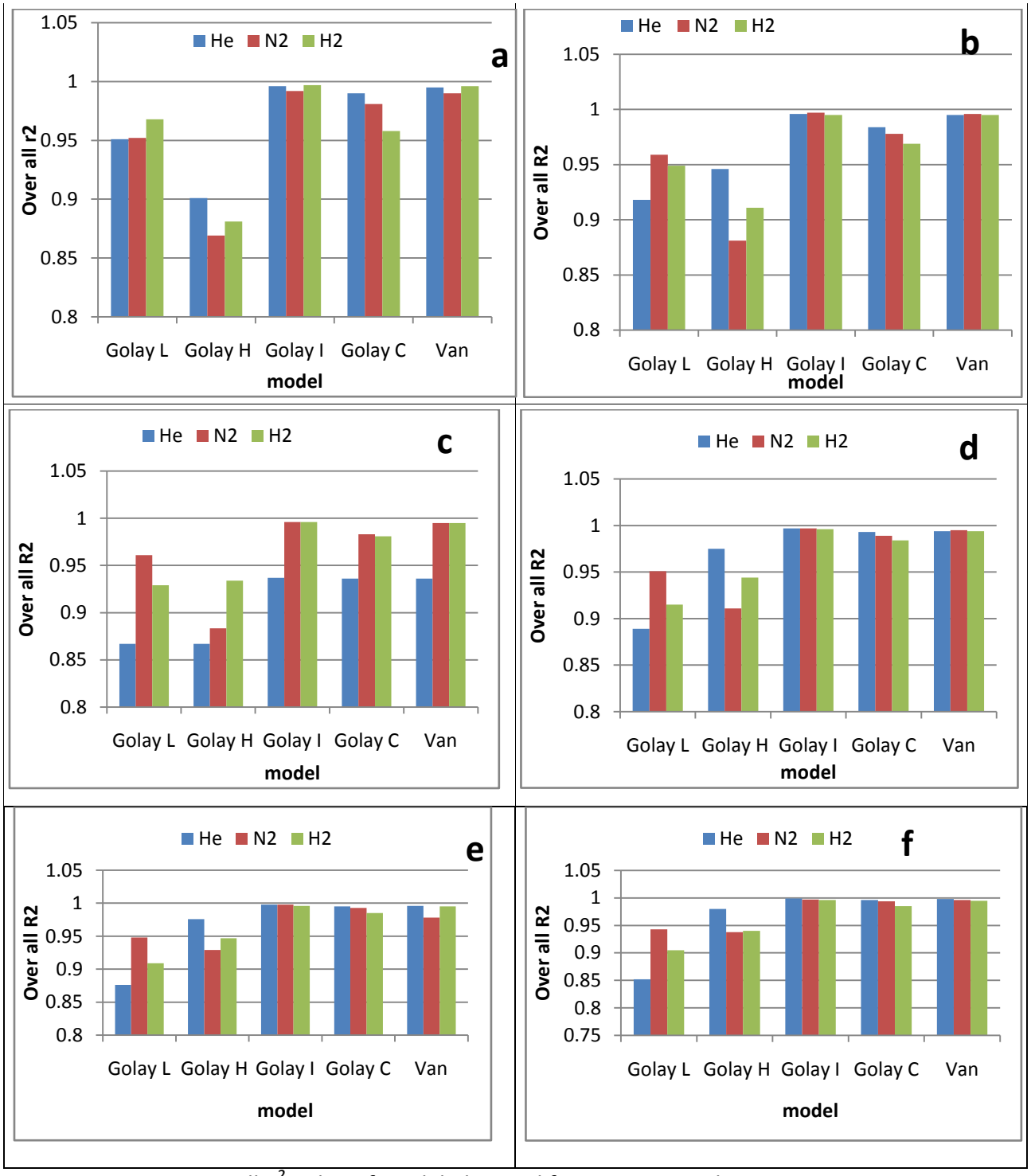

Figure 31: Over all R^2 value of model obtained from He, N_2, and H_2 as carrier gas in column lengths (a) 10m (b) 20m (c) 30m (d) 40m (e) 50m (f) 60m

Golay L- low pressure, H- high pressure, I- intermediate pressure, C- C1 &C2 (diffusion of analyte between two phases), and Van- Van Deemter

Table 10 (A, B): Golay models in three carrier gases and different column dimension

A)

	Hydrogen			Helium		
Column length(m	Model	R^2	RMSE	Model	R^2	RMSE
10	Golay (low PD)	0.968	0.0145	Golay (low PD)	0.951	0.018
	Golay (high PD)	0.881	0.058	Golay (high PD)	0.901	0.054
	Golay (interm. PD)	0.997	0.003	Golay(interm. PD)	0.996	0.0039
	Golay (C1 &C2)	0.958	0.0144	Golay (C1 &C2)	0.99	0.0143
	Van Deemter	0.996	0.004	Van Deemter	0.995	0.004
20	Golay (low PD)	0.949	0.0212	Golay (low PD)	0.949	0.0314
	Golay (high PD)	0.911	0.0531	Golay (high PD)	0.911	0.0452
	Golay (interm. PD)	0.995	0.0052	Golay(interm. P)	0.995	0.006
	Golay (C1 &C2)	0.969	0.0146	Golay (C_1 &C_2)	0.969	0.0128
	Van Deemter	0.995	0.0054	Van Deemter	0.995	0.007
30	Golay (low PD)	0.929	0.0275	Golay (low PD)	0.867	0.0396
	Golay (high PD)	0.934	0.0474	Golay (high PD)	0.867	0.0519
	Golay (interm. PD)	0.996	0.0054	Golay (interm. P)	0.937	0.0203
	Golay (C1 &C2)	0.981	0.0132	Golay (C_1 &C_2)	0.936	0.0224
	Van Deemter	0.995	0.006	Van Deemter	0.936	0.0209
40	Golay (low PD)	0.915	0.0309	Golay (low PD)	0.889	0.0424
	Golay (high PD)	0.944	0.0431	Golay (high PD)	0.975	0.0329
	Golay (interm. PD)	0.996	0.006	Golay (interm. D)	0.997	0.0061
	Golay (C1 &C2)	0.984	0.0127	Golay (C_1 &C_2)	0.993	0.0104
	Van Deemter	0.994	0.007	Van Deemter	0.994	0.008
50	Golay (low PD)	0.909	0.0312	Golay (low PD)	0.876	0.0418
	Golay (high PD)	0.947	0.0391	Golay (high PD)	0.976	0.0278
	Golay (interm. PD)	0.996	0.0057	Golay(interm. PD)	0.998	0.004
	Golay (C1 &C2)	0.985	0.0119	Golay (C1 &C2)	0.995	0.008
	Van Deemter	0.995	0.0062	Van Deemter	0.996	0.006
60	Golay (low PD)	0.905	0.0295	Golay (low PD)	0.852	0.040
	Golay (high PD)	0.94	0.0359	Golay (high PD)	0.98	0.022
	Golay (interm. PD)	0.996	0.0056	Golay(interm. PD)	0.999	0.003
	Golay (C1 &C2)	0.985	0.0113	Golay (C1 &C2)	0.996	0.006
	Van Deemter	0.995	0.0058	Van Deemter	0.998	0.004

B)

Column length (m)	Nitrogen		
	Model	R^2	RMSE
10	Golay (low PD)	0.952	0.0153
	Golay (high PD)	0.869	0.0476
	Golay (interm. PD)	0.992	0.0052
	Golay (C1 &C2)	0.981	0.0083
	Van Deemter	0.99	0.0055
20	Golay (low PD)	0.959	0.0141
	Golay (high PD)	0.881	0.0454
	Golay (interm. PD)	0.997	0.0032
	Golay (C1 &C2)	0.978	0.0097
	Van Deemter	0.996	0.0033
30	Golay (low PD)	0.961	0.0148
	Golay (high PD)	0.8835	0.0429
	Golay (interm. PD)	0.996	0.004
	Golay (C1 &C2)	0.983	0.009
	Van Deemter	0.995	0.0042
40	Golay (low PD)	0.951	0.018
	Golay (high PD)	0.911	0.038
	Golay (interm. PD)	0.997	0.004
	Golay (C1 &C2)	0.989	0.008
	Van Deemter	0.995	0.005
50	Golay (low PD)	0.948	0.019
	Golay (high PD)	0.929	0.033
	Golay (interm. PD)	0.998	0.004
	Golay (C1 &C2)	0.993	0.007
	Van Deemter	0.978	0.004
60	Golay (low PD)	0.943	0.02
	Golay (high PD)	0.938	0.03
	Golay (interm. PD)	0.997	0.004
	Golay (C1 &C2)	0.994	0.006
	Van Deemter	0.996	0.0041

5.9 Isothermal GC in Golay interim pressure drop model

Golay interim pressure drop model is good fit model regardless of column length and carrier gas type (fig.30 and 31). The model was used to study separation quality in the isothermal Gas chromatography. The generated average efficiency data from the model is presented in table 11.

Fig.32 and table 11 show that irrespective of carrier gas and length, the minimum plate height is around 0.21, with hydrogen marginally higher and nitrogen marginally lower than helium. But the results for the 10 m column deviates from the other by having higher H. The plate heights shown are corrected for the deviation between nominal and actual column lengths (1.2 m).

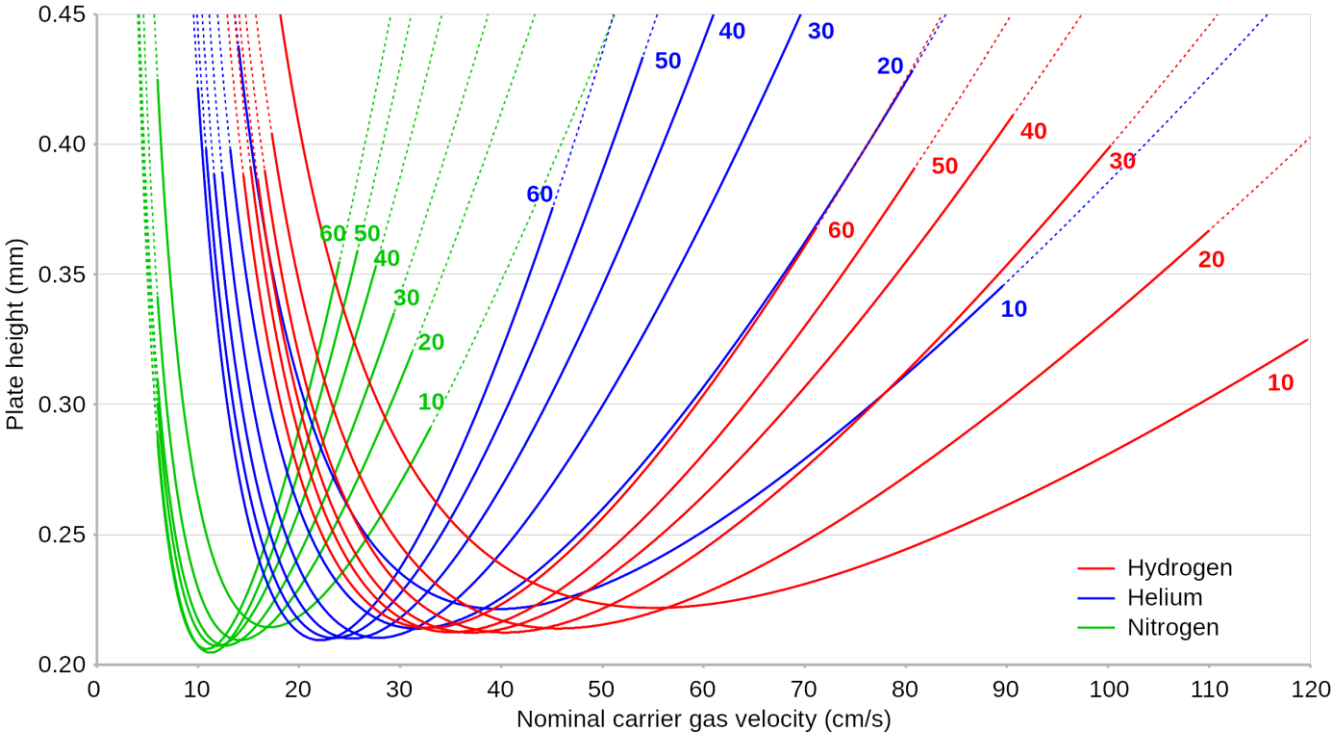

Figure 32: All models for the isothermal experiments based on Equation x ($H=B/u^x+Cu^x$). Dotted lines show extrapolated regions. The plate height is corrected for the difference between actual and nominal column lengths.

Things which are appeared in fig. 33 are generally as expected. The value of B in as equation 16 is much lower for N_2 than for the two other gases, and the value of C is much higher. B is directly proportional to the diffusion coefficient D_M of the analytes in the mobile phase (equation 18); the diffusion power of hydrogen and helium is stronger than what does nitrogen and that is why the value of B is much lower in nitrogen. Obviously mass transfer (equilibration time) is function of k, d_c, d_f, D_S, D_M (equation 18**)** so that the effect of column dimension for three of them (carrier gases) is same. It's being viscous and not easily compressible leads to have long equilibration time of analyte in between the stationary phase and mobile phase. That is the driving force to get higher value of C when nitrogen is as carrier gas. As expected, x, also

increase with column length. The optimum velocity is decreased with increasing of column length for all carrier gas (fig. 33.d). This is because when the column length and inlet pressure are directly proportional each other; This leads to shift the optimum gas speed to lower values (equation 15). Still the value of optimum velocity of nitrogen is the lowest value since its diffusivity property is too low. Fig.33, e, shows the minimum plate height and show that the values for the 10 m column is much higher. In general speaking, For fig. 33, the effects are coming from inlet pressure, nature of carrier gas and column length and other extra column effects.

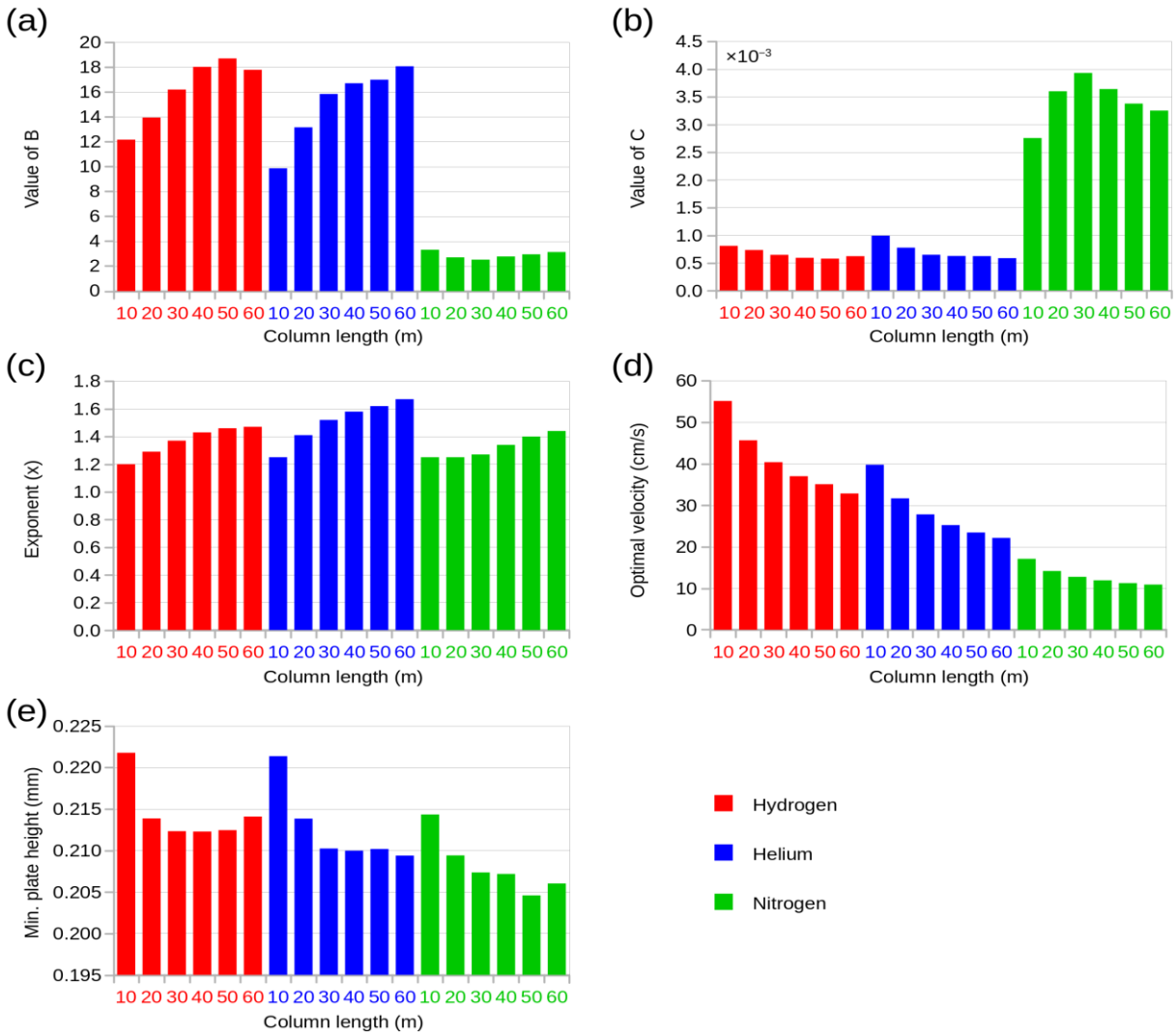

Figure 33: Differences between the models based on Equation x ($H=B/u^x+Cu^x$). (a) the size of B, (b) the size of C, (c) the size of the exponent, x, (d) optimal carrier gas velocities, and (e) minimum plate height. The minimum plate height is corrected for the difference between actual and nominal column lengths. Other parameters are not corrected.

The cause of the higher H for the 10 m column can be that the first peaks are so narrow that they get influenced by extra column effects. This is supported by the plot below (fig.34), where the individual models are compared for alkanes with He and 10, 30 and 60 m columns. At 30 and 60 m there are clear trends in the plots with the highest optimal velocities and the lowest plate heights for the shortest alkanes. This is what we should expect (smaller molecules have higher diffusion). But at 10 m we see deviations from this pattern with higher than expected values for C_{14} and C_{15}.

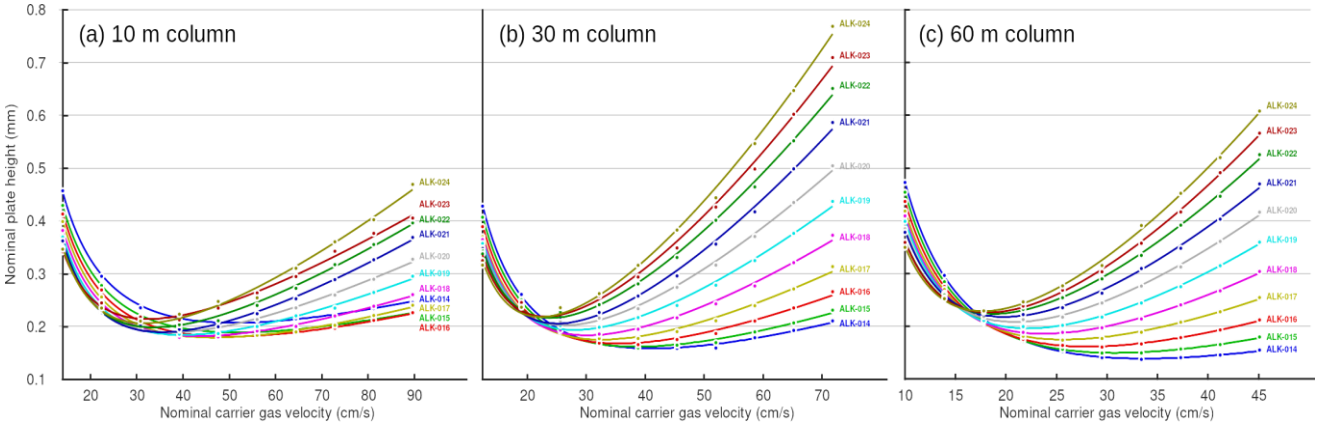

Figure 34: Individual models for alkanes with Helium as carrier gas, (a) 10 m column, (b) 30 m column, (c) 60 m column.

5.10 Comparison of column performance in isothermal condition

The performance of separation columns can be evaluated by considering their efficiency, N, and plate height, H, and plate duration (H/u). The minimum average plate height and plate duration are illustrated at the average optimum carrier gas velocity; for each carrier gas in each column length, the values of H_{actual} and H/uopt are described in fig. 33 and table 11.

As clearly observed from the fig.35, during the transition of every 10m, 30 m to 40m is the condition where maximum efficiency is improved by 4.1%. Helium is used as a carrier gas. For hydrogen and nitrogen, 10m to 20m transition is where the efficiency is increased by 3.6% and 2.3% respectively. Averagely, 10m to 20m transition is the shifting in which we may find a better improvement of separation.

Meanwhile the plate duration is able to be determined as plate height per optimum carrier gas velocity (H/u_{opt}). And hence, the plate duration of the transition, of 10 m to 20 m, 20 m to 30 m, 30 m to 40 m, 40 m to 50 m and 50 m to 60 m, for all carrier gases, is increased by 18%, 12%, 8%, 6% and 6% respectively. Maximum change of efficiency is observed in short column transition while in long transition the change is not as such substantial important.

The effect of carrier gas transition in the performances of different column length is assessed within. As described in the fig.33, the transition of single carrier gas type in all columns, there is no as such big observable change of efficiency (plate height) and plate duration time through the entire column lengths. Whereas when we replaced one carrier gas by the others, for same column length, there will be difference in both efficiency and plate duration. Helium is chosen as reference which shared properties in between nitrogen and hydrogen. By changing the carrier gas, from helium to hydrogen, helium to nitrogen, to see the effect of switching of mobile phase in the separation quality and provided as follow. And therefore, for all employed column lengths (10m-60m), replaced helium by hydrogen, nitrogen by helium the efficiency will be improved by less than 2.25%, decreased by 5.3% respectively. On the contrary, the time required for the analysis, for the above switching on carrier gases, are decreased by 31 % and increased by 111%.

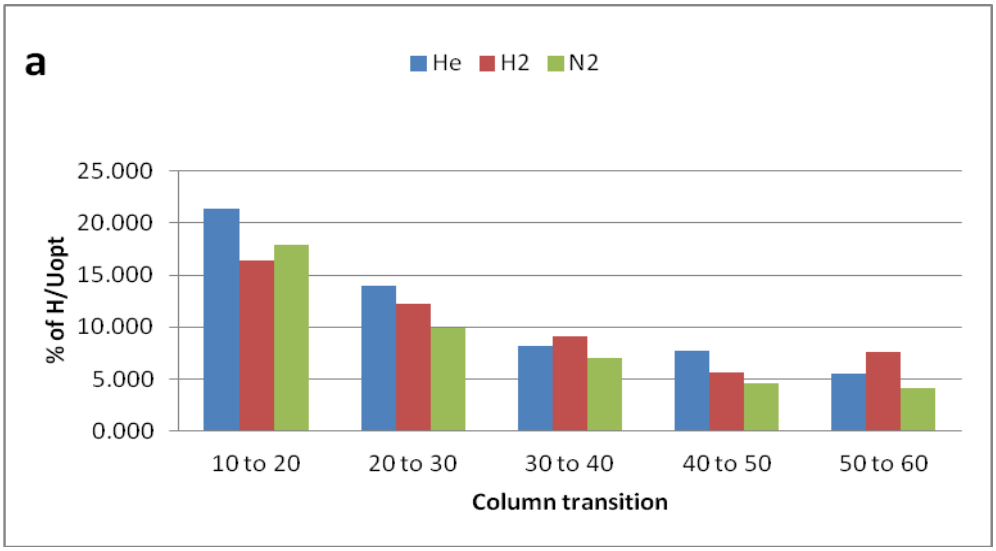

Figure 35:column transition effect

Table 11. mean VD models calculated from 10- 60m column length (column 4, Helium, hydrogen and nitrogen as carrier gas) in isothermal gas chromatography.

Carrier gas	Terms	Nominal Column length (m)					
		10	20	30	40	50	60
He	B	9.86765	13.16485	11.06164	16.70326	16.98814	18.06972
	C	0.00099	0.00077	0.00100	0.000622	0.00062	0.00058
	x	1.25000	1.41000	1.39000	1.58000	1.62000	1.67000
	Opt.Vel. (cm/s)	39.76359	31.65705	28.45939	25.21025	23.42518	22.11592
	Min. H (mm)	0.19765	0.20174	0.21061	0.20386	0.20525	0.20527
	Corr.H (mm)	0.22136	0.21384	0.21903	0.20998	0.21018	0.20937
	Plate duration (min)	0.00056	0.00068	0.00077	0.00083	0.00090	0.00095
H_2	B	12.16960	13.94508	16.19681	18.02278	18.69577	17.78413
	C	0.00081	0.00073	0.00064	0.00059	0.00058	0.00062
	x	1.20000	1.29000	1.37000	1.43000	1.46000	1.47000
	Opt.Vel.	55.12527	45.64566	40.38381	37.01541	35.08240	32.83801
	Min. H	0.19801	0.20176	0.20415	0.20609	0.20746	0.20989
	Corr.H	0.22177	0.21386	0.21232	0.21228	0.21244	0.21409
	Plate duration (min)	0.00040	0.00047	0.00053	0.00057	0.00061	0.00065
N_2	B	3.32253	2.71023	2.52732	2.77844	2.95446	3.13697
	C	0.00276	0.00360	0.00393	0.00364	0.00338	0.00325
	x	1.25000	1.25000	1.27000	1.34000	1.4000	1.44000
	Opt.Vel.	17.08033	14.14777	12.75091	11.90251	11.23763	10.87124
	Min. H	0.19137	0.19755	0.19937	0.20113	0.19979	0.20198
	Corr.H	0.21434	0.20940	0.20734	0.20716	0.20458	0.20601
	Plate duration (min)	0.00125	0.00148	0.00163	0.00174	0.00182	0.00190

Corr. H, corrected plate height which is calculated as: H corr. = $H_{actual} = H_{nominal} * L_{actual}/L_{nominal,}$

Plate duration = corr. H/ optimum velocity

5.11 Comparision of isothermal and programmed temp. GC

In isothermal GC, the performance of separation columns is evaluated by plate height, H, and plate duration (H/u_{opt}) while in programmed temperature GC, PPC and t_{topt} are used to know the performance of separation columns. The transition effect data for both column condition is presented in table 12. As claimed in table 12, the transition effect is decreased from short column to long column for both oven temperature. Still the effect of switching of carrier (He-N_2 and He-H_2) is same pattern. Magnificent change of column transition effect (both efficiency and analysis time) is being happened in programmed temperature GC than isothermal does. On the other hand, carrier gas transition effect (analysis time) in isothermal is much more significance than programmed temperature but the efficiency is not away from programmed. In sum up, significance transition effect is observed in programmed temperature GC.

Table 12. transition efficiency and analysis time in two column temperature condition

Condition	Transition condition	Programmed temp. GC			Isothermal GC		
		% PPC change	% t_{topt}	PPC/$t_{(topt)}$	% H change (decreased)	% of H/u_{opt} (Q)	H/Q
CL	10 -20m	35	104	0.34	<4.1	18	0.23
	20- 30m	19	52	0.37	4.1	12	0.34
	30-40m	13	35	0.37	<4.1	8	0.52
	40-50m	10	26	0.39	<4.1	6	0.69
	50-60m	8	21	0.38	<4.1	6	0.69
Carrier gas	He-N_2^{**}	16	10	1.60	5.3	111	0.05
	He-H_2^{*}	7	5	1.40	2.3	31	0.07

NB. ** - decrease efficiency, * decreasing time , CL – column length , Q- plate duration

5.12 Quality control

5.12.1 Cutting and installation of the column

The column cage had a diameter of 15.7 cm, which means that the circumference was 49.3 cm. According to the manufacturers specification the column was approximately 61 m when purchased (nominal length plus two coils). Two coils was removed at the first installation (60 m). For each installation a section of 9.5 m was removed (measured by laser ruler) and an additional 50 cm was removed by installation (measured by ordinary ruler). This procedure was followed for nominal lengths of 50, 40, 30, 20 and 10 m. At the end, the residual length of the column after cutting off the last 9.5 m section, was measured to be 1.7 m. This means that the column length of the last experiments (nominal 10 m) was 11.2 m (9.5 m + 1.7 m).

The mass of the column, including cage, was measured to be 23.50 g at arrival. After all the experiments were conducted the mass of the cage was measured to be 17.77 g, which means that the mass of the column initially was 5.72 g. The

masses of all the 9.5 m sections that was removed at each installation was weighed and found to be 0.8742±0.0012 g, which means that the mass per meter column was 0.09202 g. Dividing the mass of the column material (5.72 g) by the mass per meter gives an estimated initial length of 62.2 m. When approximately 1 m (two coils of 49.3 cm) was removed by installation, this leaves a column length of approximately 61.2 m for the first experiments with the nominal length of 60 m.

Since both the estimated deviation for the first experiments (60 m) and the measured deviation for the last experiment (10 m) was 1.2 m it is assumed that the column was always 1.2 m longer than the nominal dimensions. This gives an error of 12%, 6.0%, 4.0%, 3.0%, 2.4% and 2.0% for the column sections with nominal lengths of 10, 20, 30, 40, 50 and 60 m, respectively. In conclusion, the error was not bad on the quality of model.

5.12.2 Comparison of the first and last 9.5 m sections

Column cost is directly related to column length. Doubling column length nearly doubles the price of the column. When efficiency is increased by lengthening the column, there is a significant increase in column cost and the analysis time will increase. To invest the separation column with appropriate cost, efficiency and time short column length is reasonable option. Though still shorter columns cost more per meter than longer columns. Cutting longer columns into shorter lengths seems like a good method to keep safe in terms of cost, what we did is cutting longer (60m) into shorter pieces. i.e. the above column lengths. During the cutting of short column length more measuring error is observed while in longer column length the error is six times lower than the short one. The generation of more error in short column length is the cumulative effect from the consecutive cutting of column length, personal error, errors from the measuring ruler. In general speaking, the probability of individual piece variation is higher when shorter pieces are cut from the original column. Finally, there is the increased chance of tubing breakage while rewinding the shorter columns on other cages; this brings a problem on the quality of separation. Samples were run using first and second cut off 9.05m column length with same carrier gas velocity, flow, pressure and temperature. For both cut off section, the retention factor (values from nominal dead time, dead time based on regression), plate height, optimum velocity and van Deemter terms are illustrated in fig.36. There is un stability of retention factor, it is expected since the column length is short. The separation factor (both nominal and regression) value obtained from last section is lower than first does. The efficiency between is significantly different. So this deviation may be caused by the error generated during the cutting.

Table 13. Comparison of first 9.5 and last 9.05m cut off section in Isothermal condition at 210°C, He as carrier gas

Mean value	Value in $A_{F9.5}$	Value in $AL_{9.05}$	$(A_{F9.5}- A_{L9.05})*100/A_{L9.05}$ %
B	57.73079	46.5523	24.01277
C	0.001258	0.001182	6.38791
x	1.76	1.72	2.325581
Opt.Vel.	21.10576	21.66946	-2.60135
Min. H	0.538879	0.469151	14.86278

$A_{F9.05}$, $A_{L9.05}$ - obtained values in the first 9.5m and last cut off section of 9.05m column length respectively.

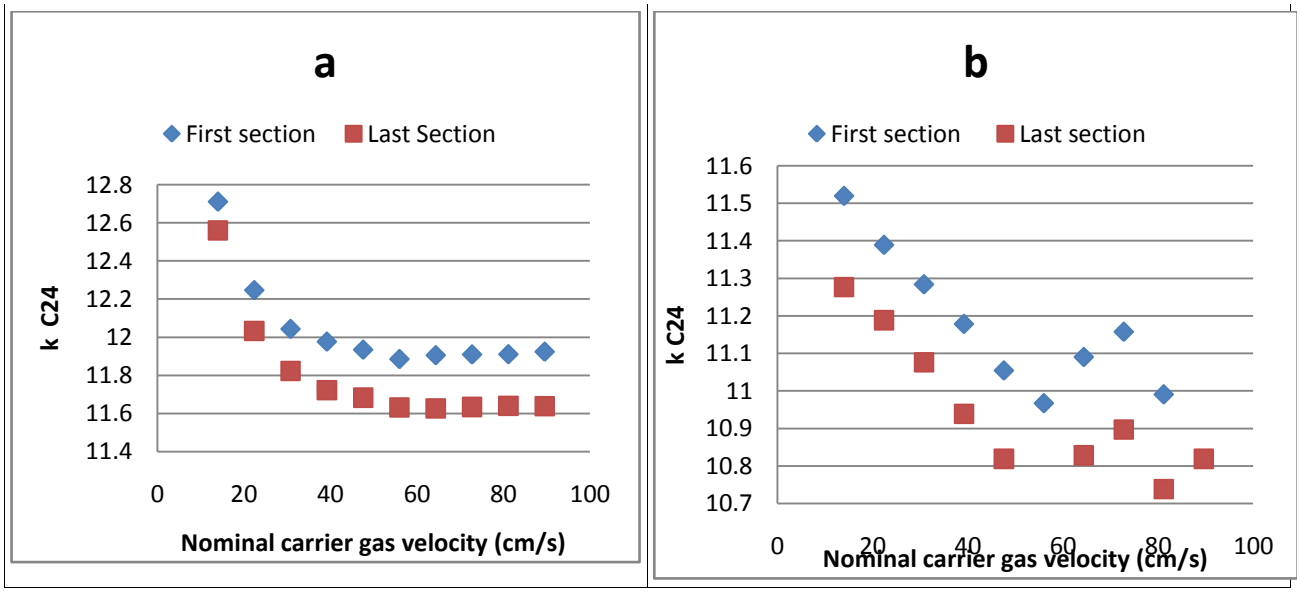

Figure 36: Retention factor of C_{24} calculated from a) t_M in cut off 9.05m column length (both in first and last cut off section) and nominal carrier gas velocity b) t_M based on regression using Chrombox.

7. Conclusion and recommendation

7.1 Conclusion

From the conducted experiments it is possible to come up with the following conclusion in gas chromatography:

- Split ratio, asymmetry, starting temperature (PTGC), oven temperature for (isothermal), sampling frequency and make up gas flow rate are very important extra column instrumental conditions which can have the potential to have contribution on the quality of separation.
- Peak widths measured in retention index units can be explained by the van Deemter equation. Similar to the height equivalent to theoretical plate (H) in the isothermal van Deemter equation the minimum the peak width is the higher the efficiency.
- In isothermal GC, dispersion models (Golay+ Van Deemter) for plate height were calculated and evaluated by their Overall R^2 and RMSE in different capillary column lengths with different level of pressure drop. Golay model at low pressure drop is good fit for Short column length. Irrespective of column dimension and carrier gas, good fit models of plate height were designed by the Golay model with interim pressure drop and Van Deemter. Van Deemter is not valid in anymore because the column is not packed column that contains multiple path effect (A).
- In programmed temperature GC, from the combination model of inverse of efficiency, W_b, RI, and retention time model the optimum time (t_{topt}), optimum velocity (u_{topt}) and peak per carbons (PPC) were obtained. The optimum temperature rate can be expressed by the relationship of PPC/t_{topt} and ratelength in each column lengths. The default optimum rate, 10°C per void time for any capillary column is proved as it is not always valid for different column length.
- The effect of carrier gas transition in the performances of different columns length is not as such big observable change of efficiency and time through the entire column lengths. Since the stationary, film thickness, column diameter is identical.
- Carrier gas type can bring a significance influence in both the time to conduct the analysis and the performance for a given separation column. The PPC in 60m, for nitrogen, is nearly equivalent to 30 m for hydrogen carrier gas and the analysis time required for hydrogen is reduced by half of the time consumed for nitrogen.
- For all carrier gases, 40 m to 50 m, 50 m to 60 m are the transition where maximum 0.4% and minimum 0.3% PPC/t_{topt} values are observed respectively.

Switching of carrier gas, helium by nitrogen gives poor efficiency with long analysis time regardless of column length.

7.2 Recommendation

In this study temperature velocity, column length factors were changing with constant column diameter, film thickness, and stationary phase. It would be pretty good column diameters are considering for further study along with the above important variables and a better model may be developed which better explains the chromatographic separation process. Since Column diameter has an influence over five parameters of primary concern in chromatography condition. They are efficiency, retention, pressure, carrier gas flow rate, and capacity.

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9. Appendix A

*Table 14. Statistical testing of starting temp. in condition of HP-5,10 m x 250µm x 0.25µm, programmed temperature **25℃/min**, He as carrier gas and its velocity is 25cm/s, constant flow rate.*

Sta. temp	peak	α-value	p-value	decision
40/60	C14	0.05	1.9420E-5	Significance different in peak width in between 40 and 60°C Since p-value < α-value
	C15	0.05	0.0451	Significance different in peak width in between 40 and 60°C Since p-value < α-value
	C16	0.05	0.04145	Significance different in peak width in between 40 and 60°C Since p-value < α-value
40/80	C14	0.05	1.27E-08	Significance different in peak width in between 40 and 80°C Since p-value < α-value
	C15	0.05	1.47E-06	Significance different in peak width in between 40 and 80°C Since p-value < α-value
	C16	0.05	0.02	Significance different in peak width in between 40 and 80°C Since p-value < α-value
60/80	C14	0.05	0.001	Significance different in peak width in between 80 and 60°C Since p-value < α-value
	C15	0.05	0.007	Significance different in peak width in between 80 and 60°C Since p-value < α-value
	C16	0.05	0.0009	Significance different in peak width in between 80 and 60°C Since p-value < α-value

Table 15. Statistical testing of starting temp. in condition of HP-5,10 m x 250µm x 0.25µm, programmed temperature 05 ℃/min, He as carrier gas and its velocity is 25cm/s, constant flow rate.

Sta.temp.	peak	α-value	p-value	decision
40/60	C14	0.05	0.0012	Significance different in peak width in between 40 and 60°C Since p-value < α-value
	C15	0.05	0.045	Significance different in peak width in between 40 and 60°C Since p-value < α-value
	C16	0.05	0.041	Significance different in peak width in between 40 and 60°C Since p-value < α-value
40/80	C14	0.05	1.268E-08	Significance different in peak width in between 40 and 80°C Since p-value < α-value
	C15	0.05	1.46919E-06	Significance different in peak width in between 40 and 80°C Since p-value < α-value
	C16	0.05	0.02	Significance different in peak width in between 40 and 80°C Since p-value < α-value
60/80	C14	0.05	0.0009	Significance different in peak width in between 60 and 80°C Since p-value < α-value
	C15	0.05	0.006	Significance different in peak width in between 60 and 80°C Since p-value < α-value
	C16	0.05	0.084 E-06	no Significance different in peak width in between 60 and 80°C Since p-value < α-value

Table 16. Statistical testing of detector frequency influence in peak width experimental conditio of (HP-5,10 m x 250µm x 0.25µm, 60 °C for 0 min, 30 ℃/min, 30 cm/s, velocity of carrier gas, He, constant flow rate, 1:200 split ratio, 40mL/min make up gas flow rate).

Detector frequency	Average peak width	α-value	p-value	Statically Decision
10Hz	0.0248	0.05	7.36E-06 (10 & 20 Hz)	Significant difference in peak width in between 10 and 20 Hz, Since p-value < α
20Hz	0.0239	0.05	0.139 (20 & 50 Hz)	No Significance difference in peak width in between 20 and 50 Hz, Since p-value > α
50Hz	0.0236	0.05	0.919 (50 & 100 Hz)	No Significance difference in peak width in between 50 and 100 Hz, Since p-value > α
100Hz	0.0236	0.05	2.68E-05 (10 & 100 Hz)	Significance difference in peak width in between 10 and 100 Hz, Since p-value < α

Table 17. split ratio effects in peak widths

Analyte	Split ratio					
	1:200	1:100	1:50	1:25	1:12.5	1:6.25
C14	0.0179	0.0191	0.0225		-	-
C15	0.0193	0.0205	0.0233	-	-	-
C16	0.0217	0.0225	0.0254	0.0353	-	-
C17	0.0256	0.0263	0.0286	0.0373	0.0654	-
C18	0.0319	0.0320	0.0337	0.0409	0.0646	-
C19	0.0403	0.0403	0.0421	0.0481	0.0685	0.1929
C20	0.0527	0.0530	0.0545	0.0587	0.0767	0.1877
C21	0.0725	0.0727	0.0745	0.0766	0.0905	0.1892
C22	0.0987	0.0999	0.1012	0.1052	0.1149	0.2003
C23	0.1392	0.1397	0.1385	0.1434	0.1494	0.2275
C24	0.1914	0.1934	0.1969	0.1962	0.1994	0.2635
Av.	0.0647	0.0654	0.0674	0.0824	0.1037	0.2102

- Peaks hidden by the solvent

Table 18. Efficiency and optimum analysis time of column length (column 4) in the same ratelength with carrier gases (H_2, He, N_2).

CL [m]	TR[°C/min]	RL[m°C/min]	hydrgen		Helium		Nitrogen	
			t(topt)	PPC	t(topt)	PPC	t(topt)	PPC
60	1	60	163.34	49.19	172.42	46.60	188.48	41.39
50	1.2	60	135.03	45.46	142.44	42.86	155.38	38.12
40	1.5	60	107.06	41.09	112.89	38.96	123.62	34.57
30	2	60	79.42	36.00	83.51	34.26	91.74	30.39
20	3	60	52.26	30.02	54.89	28.57	60.49	25.13
10	6	60	25.66	21.83	26.84	21.16	29.64	18.41
60	2	120	89.78	45.12	95.03	41.96	104.03	35.83
50	2.4	120	74.19	41.76	78.47	38.74	85.70	33.32
40	3	120	58.79	37.80	62.14	35.28	68.09	30.28
30	4	120	43.54	33.25	45.92	31.17	50.45	26.80
20	6	120	28.63	27.80	30.13	26.14	33.19	22.31
10	12	120	14.03	20.38	14.70	19.49	16.34	16.47
60	3	180	63.11	41.87	66.89	38.43	73.18	31.99
50	3.6	180	52.14	38.80	55.22	35.57	60.35	29.90
40	4.5	180	41.30	35.14	43.70	32.43	47.87	27.26
30	6	180	30.56	31.02	32.28	28.75	35.42	24.23
20	9	180	20.08	25.98	21.16	24.21	23.29	20.28
10	18	180	9.84	19.17	10.30	18.15	11.46	15.04
60	4	240	49.11	39.18	52.09	35.60	56.95	29.10
50	4.8	240	40.56	36.33	40.56	36.33	47.03	27.25
40	6	240	32.12	32.92	34.01	30.12	37.24	24.93
30	8	240	23.75	29.15	25.12	26.76	27.53	22.24
20	12	240	15.60	24.44	16.46	22.62	18.09	18.68
10	24	240	7.64	18.13	8.00	17.04	8.88	13.91
60	5	300	40.41	36.89	42.89	33.25	46.85	26.79
50	6	300	33.36	34.23	35.40	30.85	38.76	25.11
40	7.5	300	26.42	31.02	27.98	28.19	30.63	23.05
30	10	300	19.53	27.54	20.66	25.08	22.63	20.63
20	15	300	12.82	23.12	13.54	21.27	14.87	17.38
10	30	300	6.28	17.23	6.57	16.09	7.26	12.96

CL – Column length, RL- Rate Length, TR- Temperature rate, t(topt)- optimum time, PPC-efficiency

Table 19. proposed optimum temperature rate of column in different oven temperature at constant flow rate.

CL [m]	Cg	Rate [°C/min]	t_R, C24 [min]	T_{tR} [°C]	$t_{1/2}$ [min]	$T_{t1/2}$ [°C]	u_{60} [cm/s]	$u_{Tt/2}$ [cm/s]	u_{Telu} [cm/s]	$R_{opt,60}$ [10°C/t_M	$R_{opt,t1/2}$ 10°C/t_M	$R_{opt,elute}$ 10°C/t_M
60	He	3	67.3	261.9	33.6	160.9	23.1	26.7	28.5	2.3	2.7	2.8
	H2		63.5	250.5	31.7	155.2	31.9	36.0	39.1	3.2	3.6	3.9
	N2		73.6	280.9	36.8	170.4	12.5	14.6	16.1	1.3	1.5	1.6
50	N2	3.6	61.3	280.6	30.6	151.9	24.3	15.3	17.4	2.9	1.8	2.1
	H2		52.0	247.4	26.0	138.1	33.4	37.4	41.6	4.0	4.5	5.0
	He		55.3	259.1	27.7	143.0	13.2	26.6	28.9	1.6	3.2	3.5
40	He	4.5	43.4	255.4	21.7	125.1	25.9	28.1	31.3	3.9	4.2	4.7
	H2		41.0	244.6	20.5	121.5	35.3	36.9	42.5	5.3	5.5	6.4
	N2		48.3	277.4	24.2	132.5	13.7	15.5	18.3	2.1	2.3	2.7
30	He	6	32.0	252.1	16.0	108.0	28.2	30.5	34.2	5.6	6.1	6.8
	H2		30.3	241.9	15.2	105.5	38.0	41.3	49.2	7.6	8.3	9.8
	N2		35.8	274.5	17.9	113.6	14.7	16.3	20.1	2.9	3.3	4.0
20	He	9	21.1	249.7	10.5	91.6	31.6	40.0	47.4	9.5	12.0	14.2
	H2		20.0	240.0	10.0	90.0	42.0	44.7	55.9	12.6	13.4	16.8
	N2		23.6	272.5	11.8	95.4	16.1	17.5	22.8	4.8	5.2	6.9
10	N2	18	11.8	272.5	5.9	77.7	38.5	19.8	28.2	23.1	11.9	16.9
	H2		9.9	238.9	5.0	74.9	50.2	52.0	69.8	30.1	31.2	41.9
	He		10.5	248.1	5.2	75.7	18.8	39.7	51.9	11.3	23.8	27.2

tR, C_{24}- the retention time of the last eluted analyte, u_{60} – carrier gas velocity at inlet temperature (60°), $t_{1/2,}$ half time, $T_{t/2}$ – temperature at half time, u_{Telu-} carrier gas velocity at elution temperature. t_{M-} void time calculated from column length and above two carrier gas velocities. Elution temperature was calculated by equation 26.
CL- column length
Cg- carrier gas

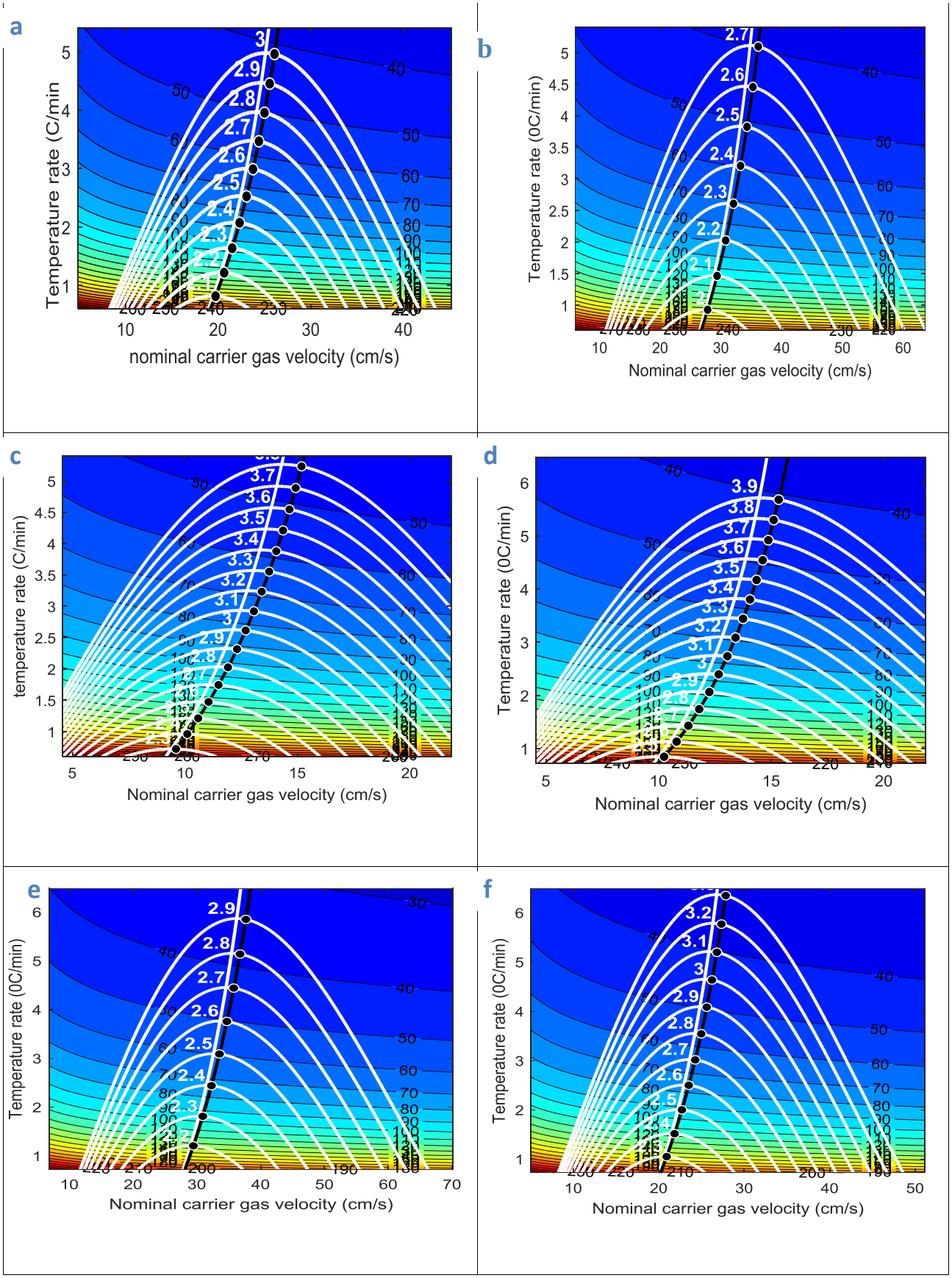

Figure 37: Eff/time obtained from 60m×250µm×0.25µm, programmed temperature condition in carrier gas (a) He (b) H_2 (c) N_2 and from 50m×250µm×0.25µm(d) N_2 (e) H_2 (f) N_2

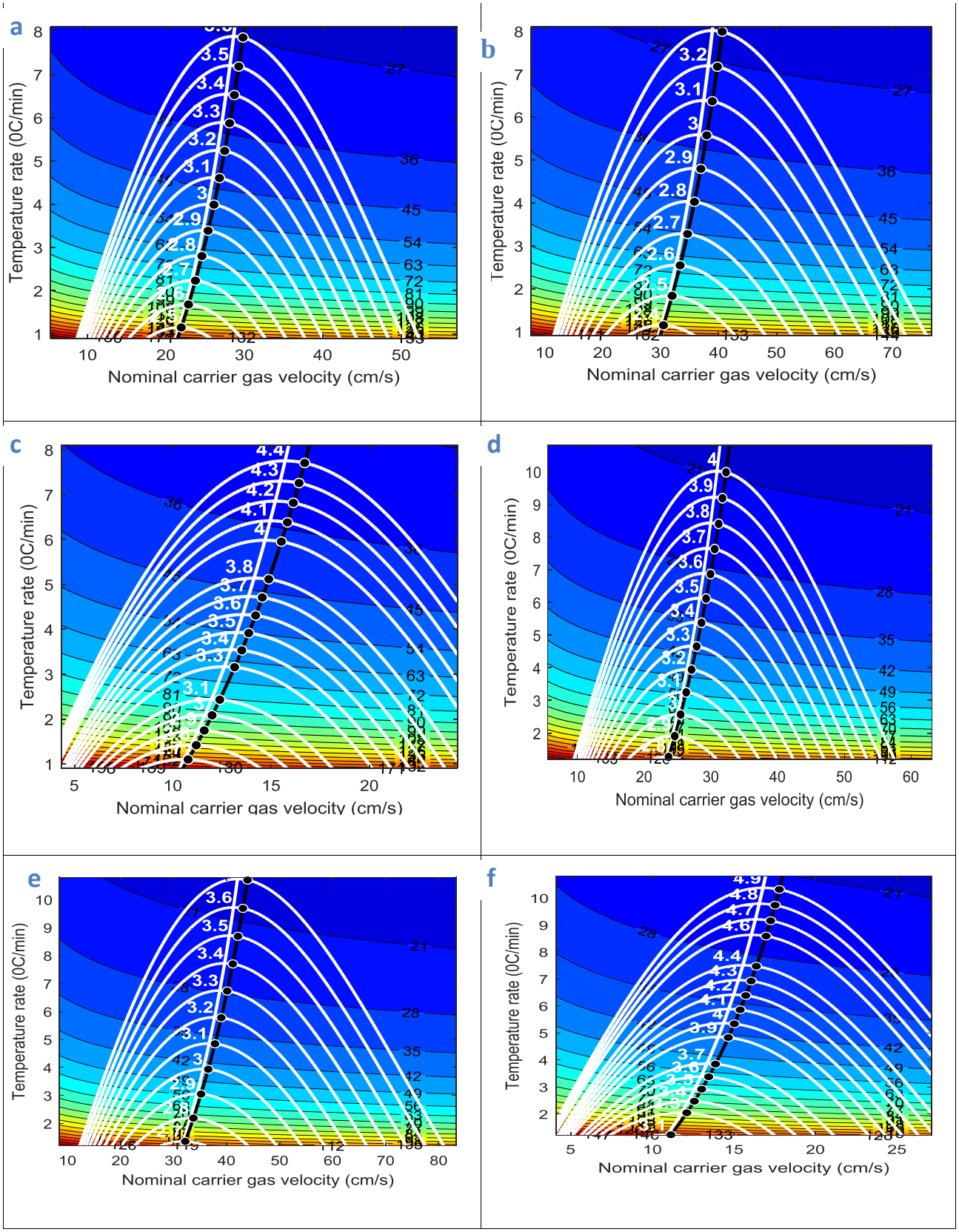

Figure 38: Eff/time obtained from 40m×250µm×0.25µm, programmed temperature condition in carrier gas (a) He (b) H_2 (c) N_2 and from 30m×250µm×0.25µm(d) N_2 (e) H_2 (f) N_2

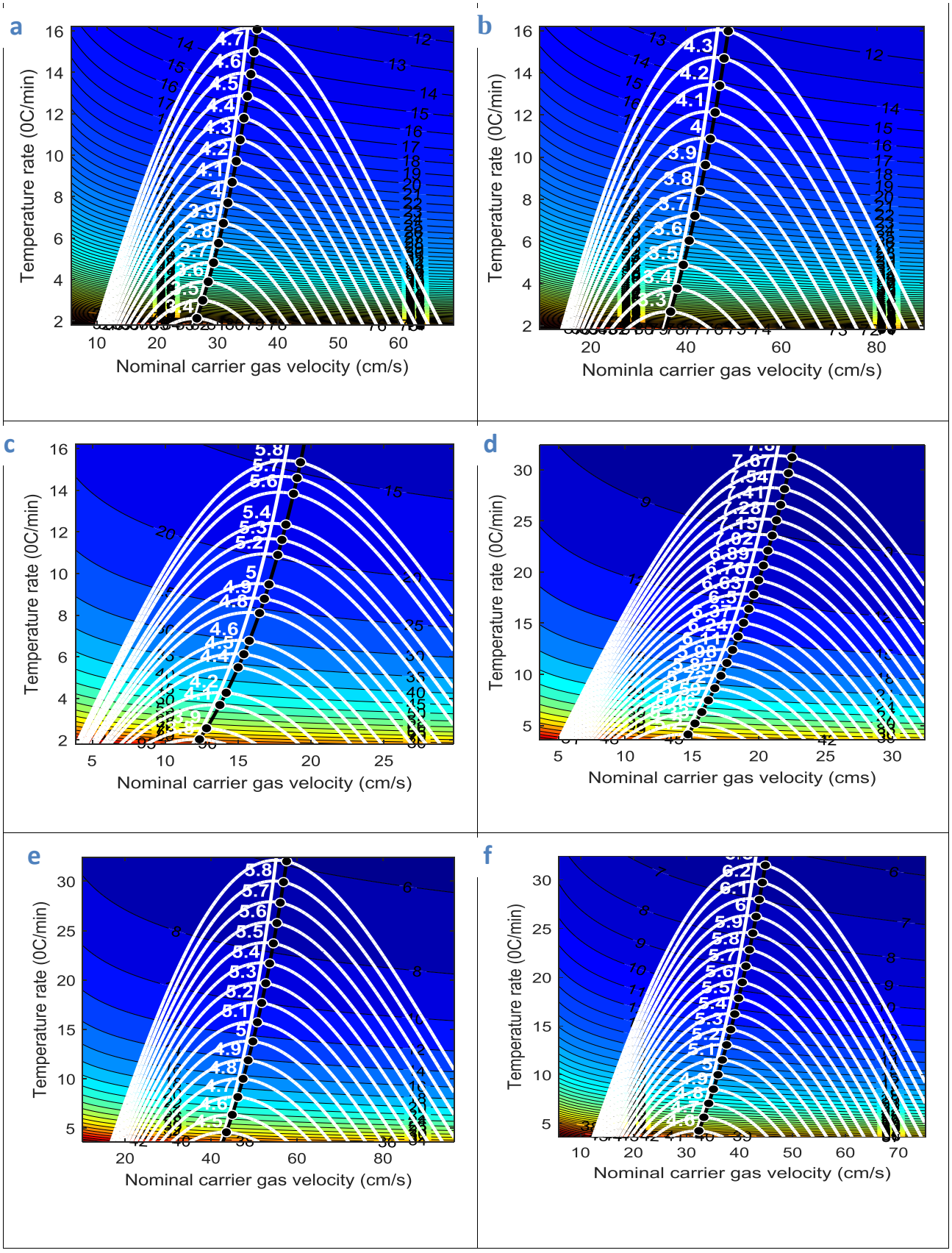

Figure 39: Eff/time obtained from 20m×250µm×0.25µm, programmed temperature condition in carrier gas (a) He (b) H_2 (c) N_2 and from 10m×250µm×0.25µm(d) N_2 (e) H_2 (f) He

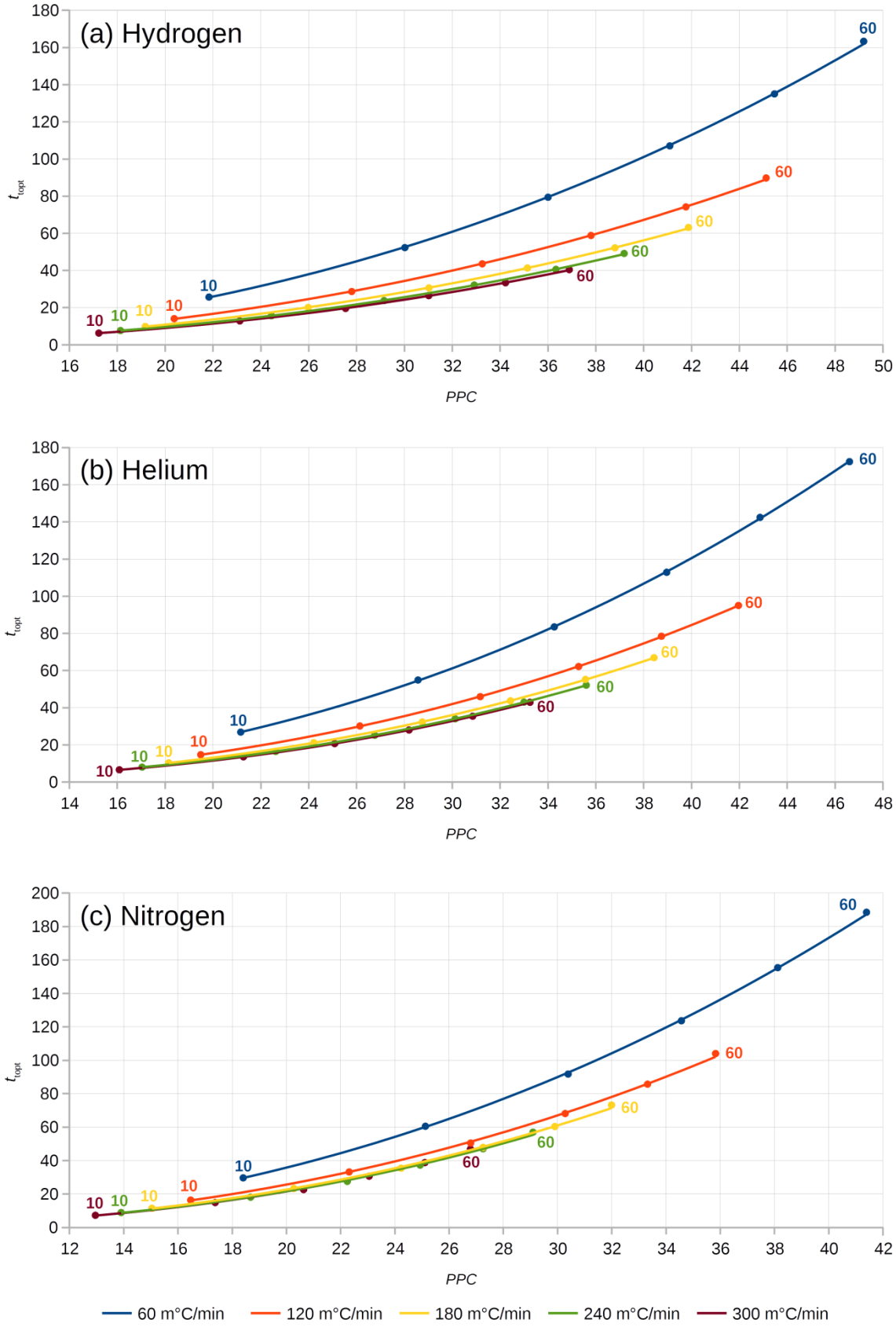

Figure 40: Different ratelengths compared for different carrier gases. Numbers on the extremes of the curves show column length in m.