GAS CHROMATOGRAPHY: INJECTION TECHNIQUES
≈ *CAPILLARY COLUMNS* ≈

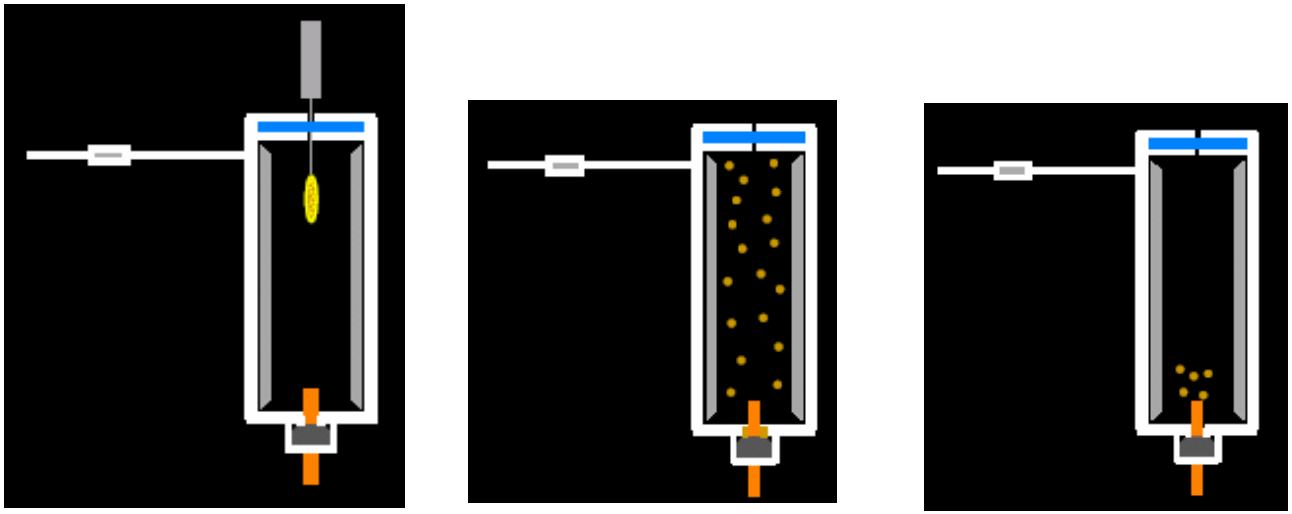
FLASH VAPORISATION INJECTION

- **Split**
- **Splitless**
- **On-Column**

COOL INJECTION – Large Volume Injection (LVI)

- **On-Column**
- **On-Column-SVE (with solvent vapour exit)**
- **PTV**

FLASH VAPORISATION INJECTION TECHNIQUES



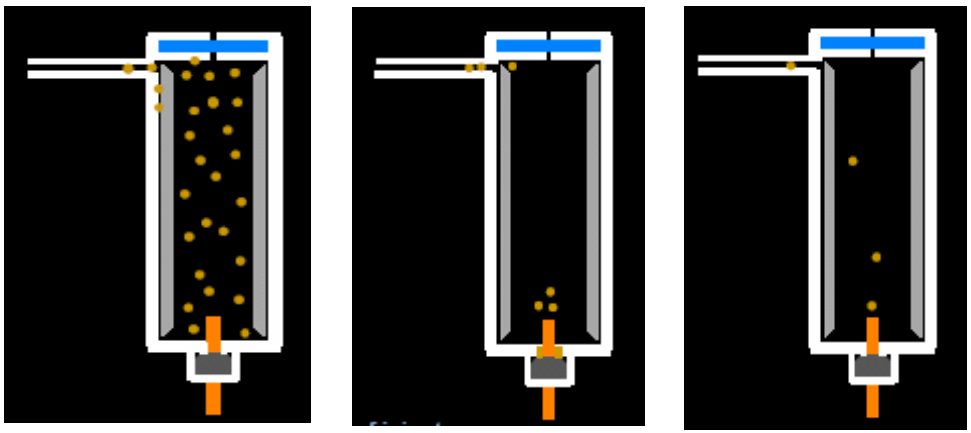
(FIGURES: Allen K. Vickers, Agilent Technologies)

RISKS: BACKFLASH and DISCRIMINATION

FLASH VAPORISATION INJECTION TECHNIQUES

BACKFLASH

- **at the vaporisation sample is expanding up to 100 – 1000 x**
- **if vapour volume > liner volume (overfill)**



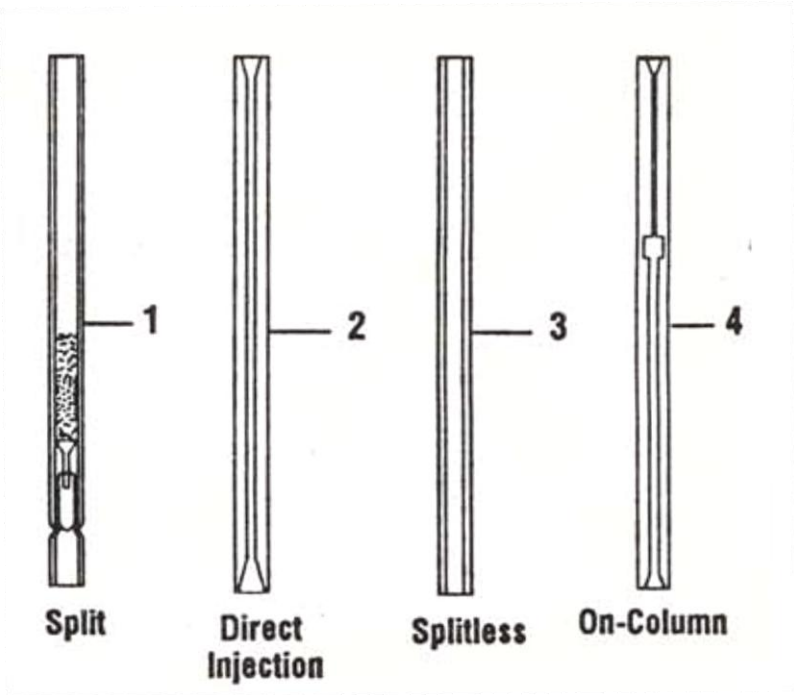
(FIGURES: Allen K. Vickers, Agilent Technologies)

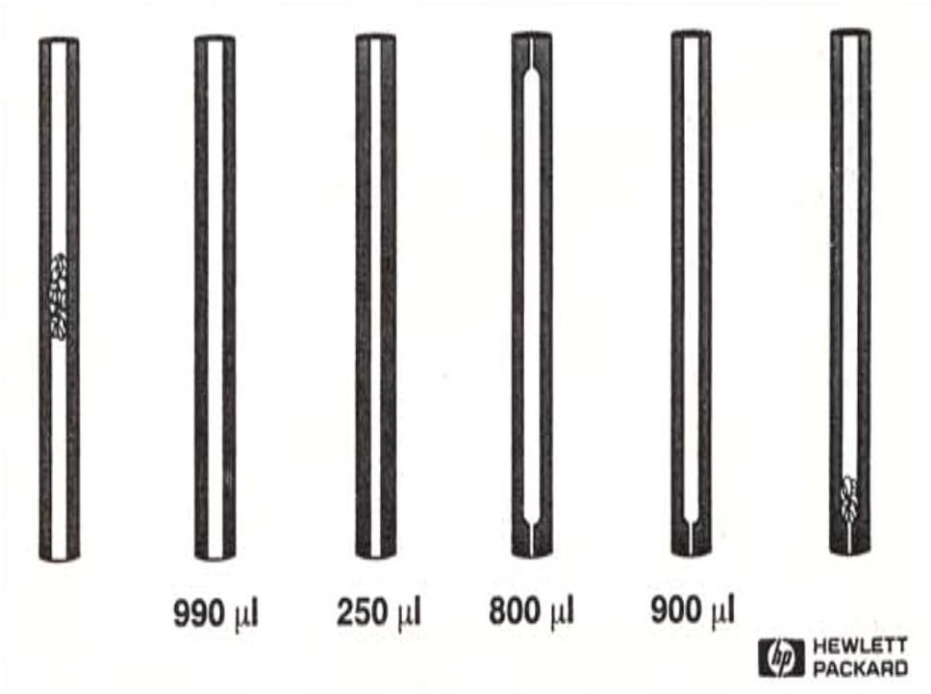
- **samples losses**
- **tailing solvent**
- **ghost peaks**

Minimization:

- ↑ liner volume
- ↓ injection volume
- ↓ expanding solvent
- ↓ injection temperature
- ↑ carrier gas flow rate
- ↑ column head pressure
- → pulsed injection

INJECTION TECHNIQUES - LINERS



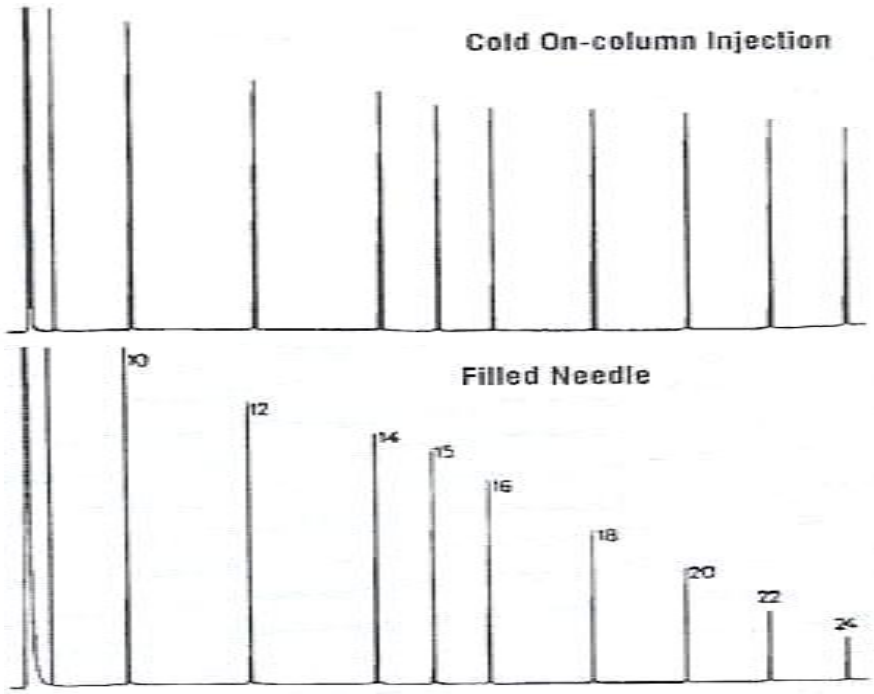


(FIGURE:Hewlett-Packard (Agilent Technologies))

FLASH VAPORISATION INJECTION TECHNIQUES

DISCRIMINATION

- injected sample ≠ sample introduced into column
- caused by different volatility of sample components
- ↑ volatility ⇒ ↑ into column



(FIGURES: Allen K. Vickers, Agilent Technologies)

Important factors:

- sample heating effectiveness
- efficiency of sample vapours mixing with mobile phase
- column position in injection chamber
- discrimination in injection chamber X in syringe
- necessary adjustment of the same conditions

INJECTION TECHNIQUES – MANUAL INJECTION

FILLED NEEDLE

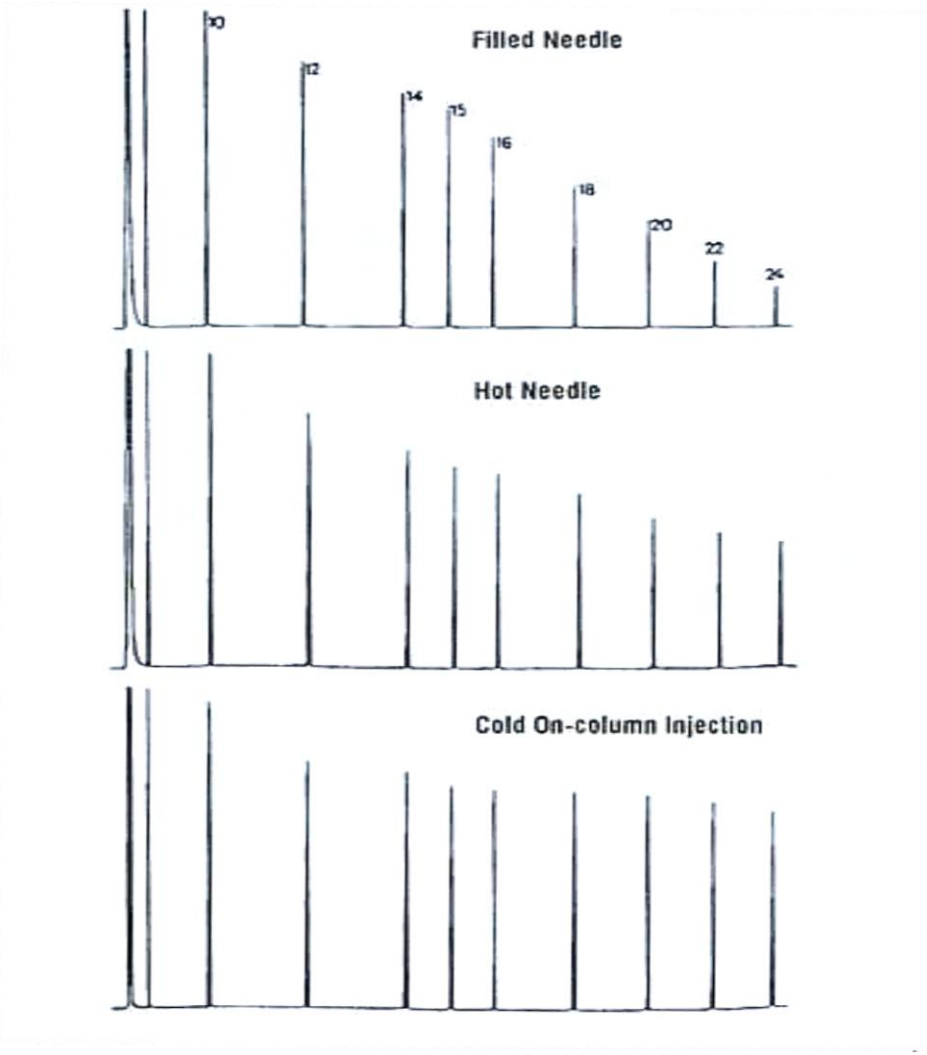
COLD NEEDLE

HOT NEEDLE *

SOLVENT FLUSH *

AIR FLUSH *

***.....non discriminative**
(in syringe)



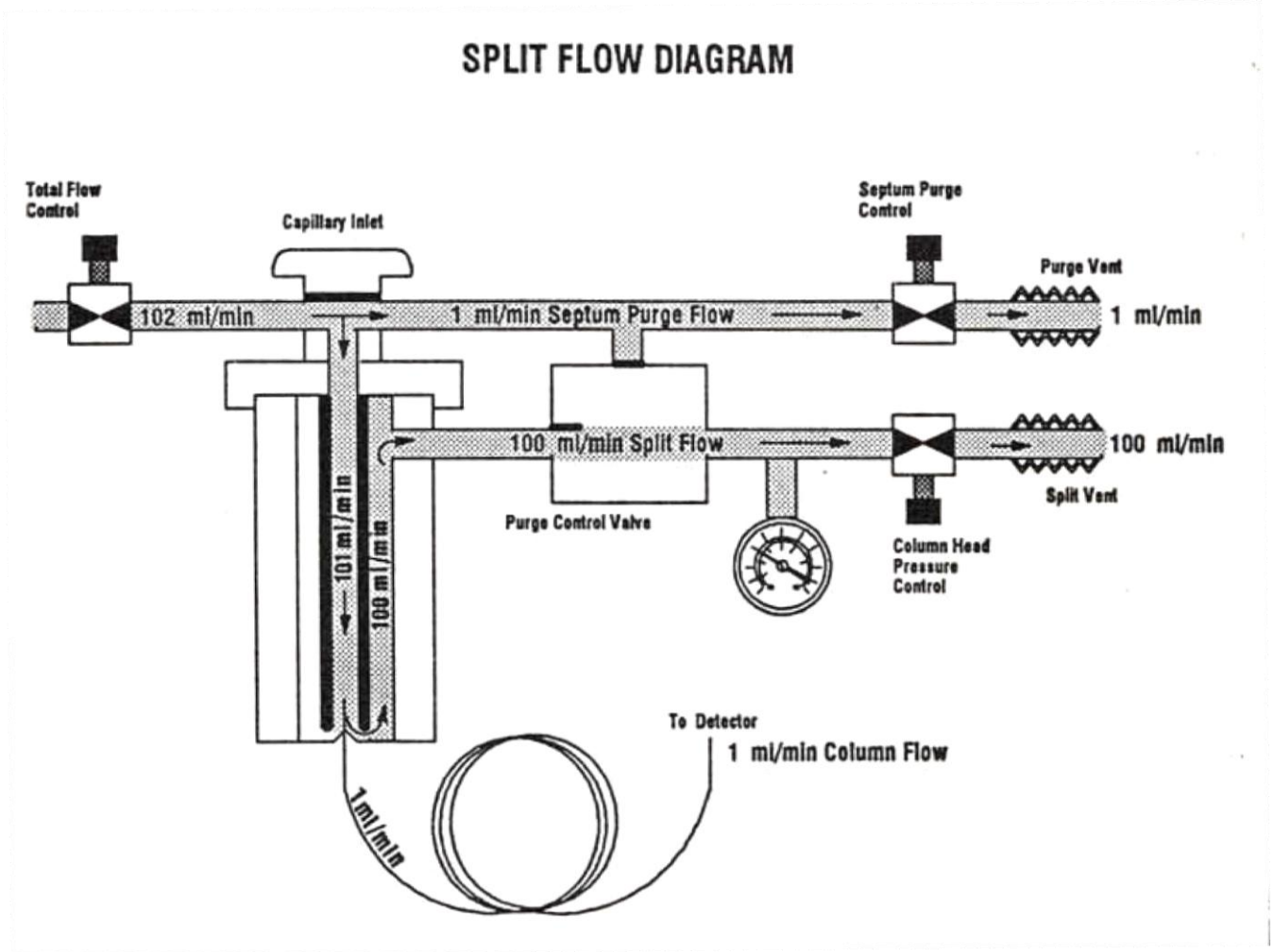
(FIGURES: Allen K. Vickers, Agilent Technologies)

INJECTION TECHNIQUES

MANUAL xxx AUTOMATIC INJECTION

	MANUAL		AUTOMATIC	
PCB	AREA	RSD (%)	AREA	RSD (%)
28	47896	12	48347	3
52	41066	5	41658	2
101	51353	7	52223	6
153	53425	14	57166	1
138	52353	18	58862	1
180	54007	23	61942	1
AVERAGE		13		2

INJECTION TECHNIQUES - SPLIT



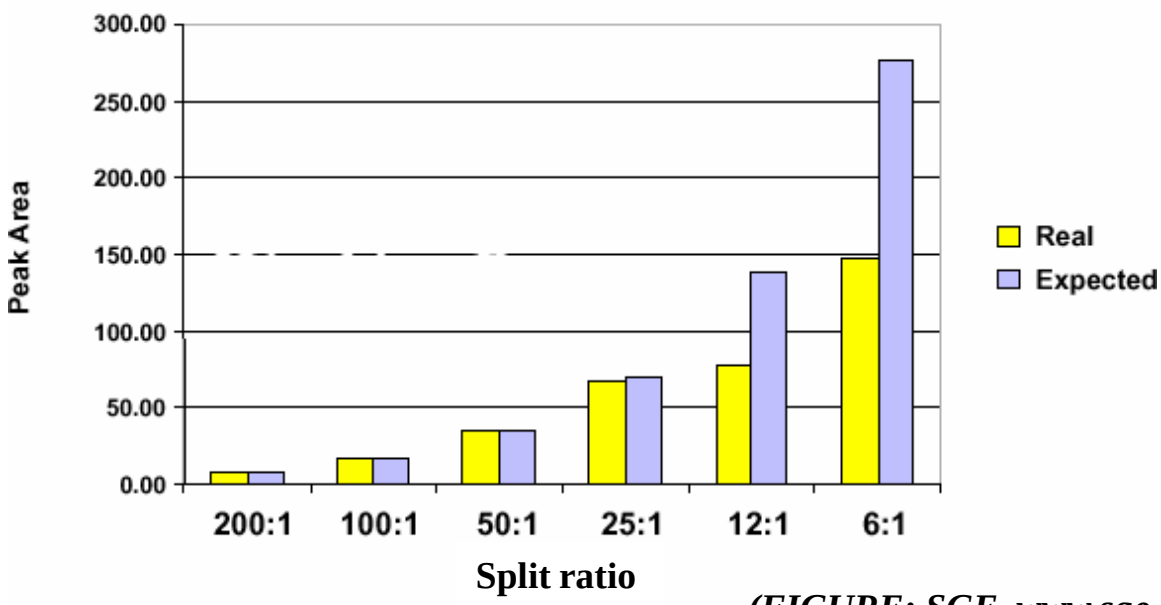
(FIGURE:Hewlett-Packard (Agilent Technologies))

INJECTION TECHNIQUES - SPLIT

SPLIT RATIO: given by column i.d. and concentration

0.1 mm:	1 : 1000	*min: 1 : 50*
0.2 – 0.32 mm:	1 : 50 - 1 : 500	*min: 1 : 10*
0.53 mm:	1 : 5 - 1 : 50	*min: 1 : 2*

- split ratio determines a sample amount introduced into column
- **split ratio ≠ sample partitioning ratio**



(FIGURE: SGE, www.sge.com)

INJECTION TECHNIQUES - SPLIT

SPLIT RATIO:

Ideal case: *sample completely in gas phase, homogeneously mixed with carrier gas*

Real case: **sample contains components with different volatility**

- incomplete evaporation

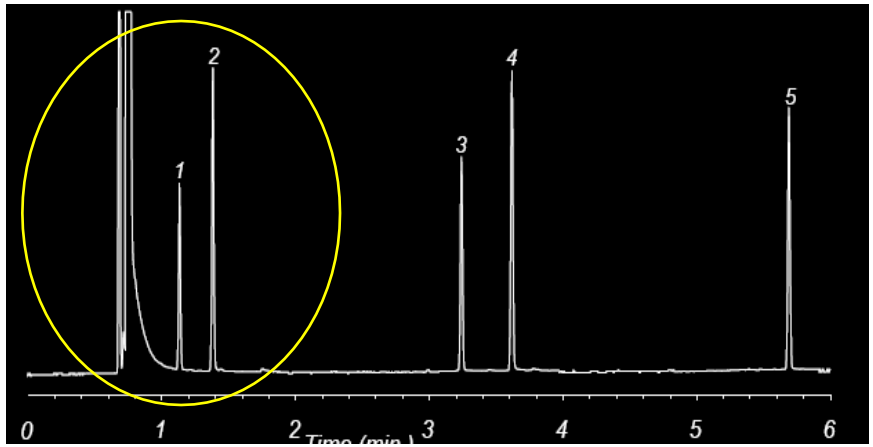
- various diffusivity of sample components

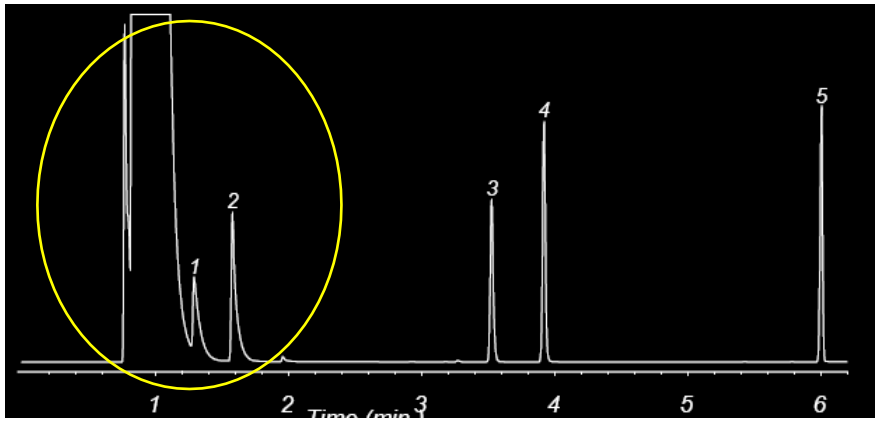
- fluctuating split ratio

= DISCRIMINATION (distorted composition)

= WORSE REPEATABILITY

INJECTION TECHNIQUES - SPLIT





SPLIT RATIO:

1 : 200

1 : 5

DB-1 (15m x 0.25mm x 0.25µm)

(FIGURE: Hewlett-Packard (Agilent Technologies))

INJECTION TECHNIQUES - SPLIT

SPLIT RATIO IS AFFECTED BY:

Sample volatility

Solvent type

Injected volume

Injection chamber volume

Injection technique

Injection temperature

Column temperature (sample re-condensation)
- **zone of decreased pressure**
- **imbibition of additional sample vapours**

INJECTION TECHNIQUES - SPLIT

REDUCING OF DISCRIMINATION:

Liners (glass wool)

Increased temperature of injection

Fast hot needle

IMPROVEMENT OF REPRODUCIBILITY:

Identical injected volume

Identical solvent

Internal standard technique

Identical starting temperature

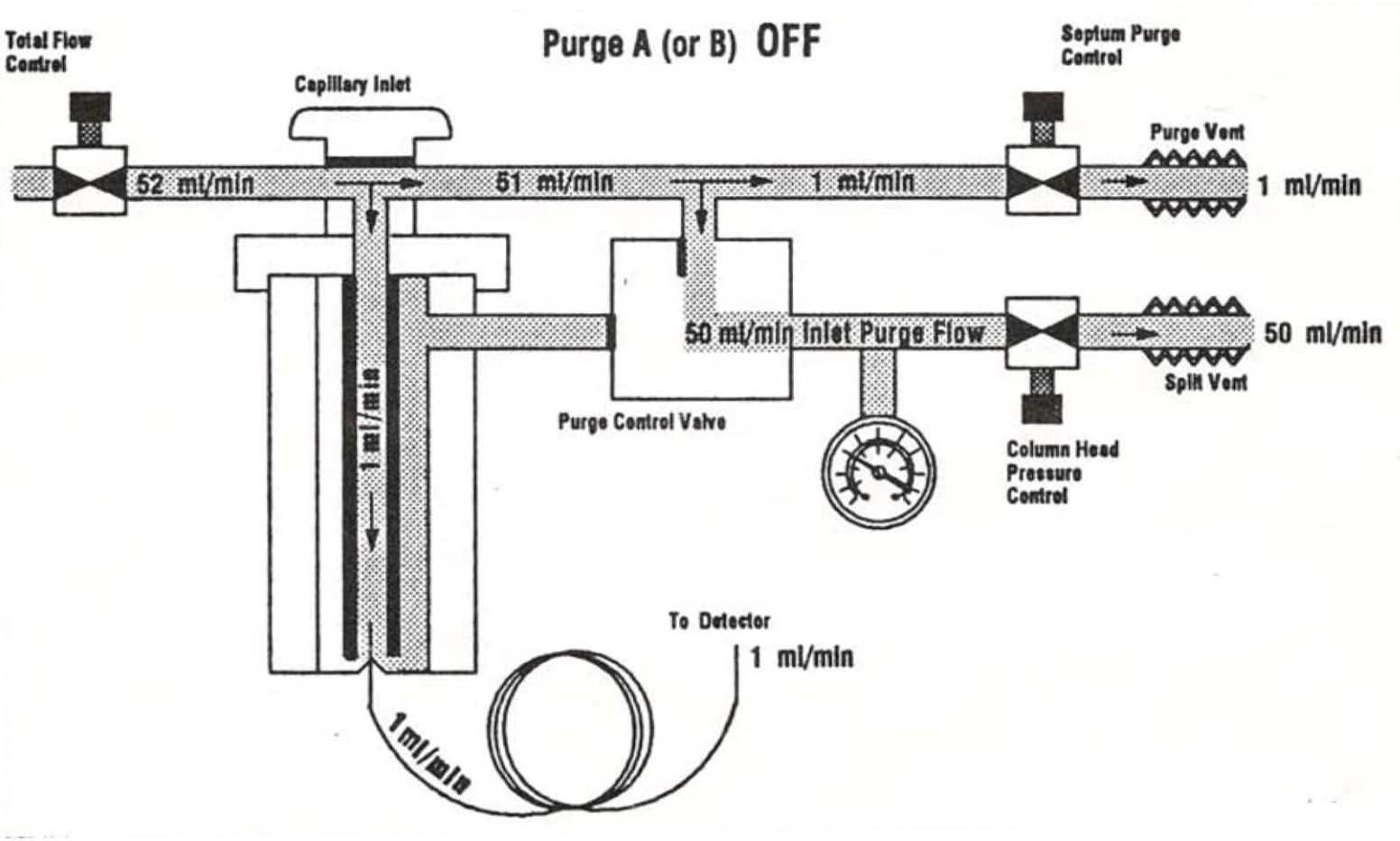
APPLICABILITY - analytes eluting before solvent

- dirty samples

- high concentration of analytes

- for columns with very small i.d.

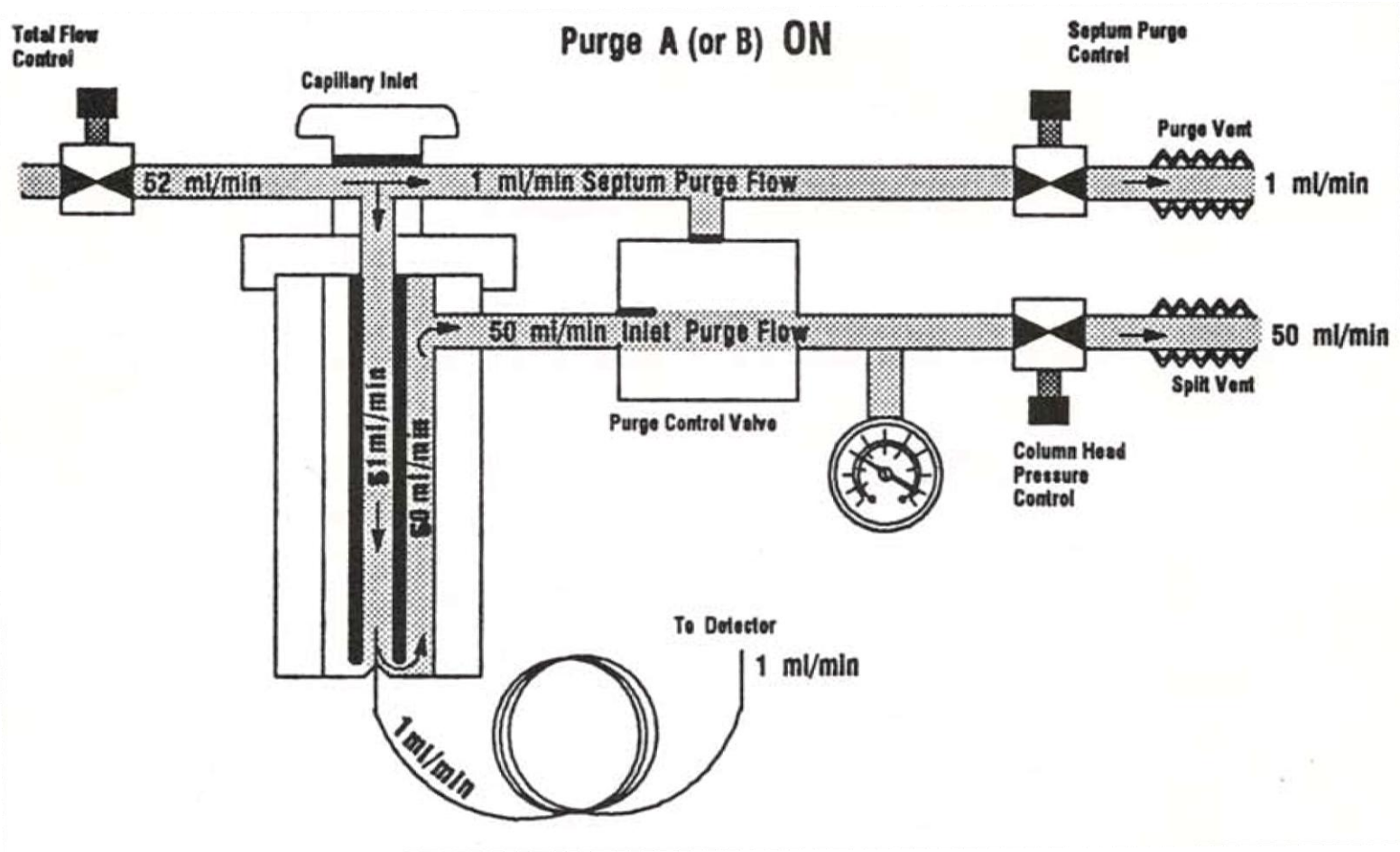
INJECTION TECHNIQUES - SPLITLESS



SAMPLE INTRODUCTION INTO COLUMN – splitless period (1)

(FIGURE: Hewlett-Packard (Agilent Technologies))

INJECTION TECHNIQUES - SPLITLESS



SAMPLE INTRODUCTION INTO COLUMN – split period (2)

(FIGURE: Hewlett-Packard (Agilent Technologies))

INJECTION TECHNIQUES - SPLITLESS

SPLITLESS PERIOD (t_s) – *experimentally, depends on:*

Solvent properties

Analytes properties

Injection chamber volume

Injected volume

Injection speed

Carrier gas speed

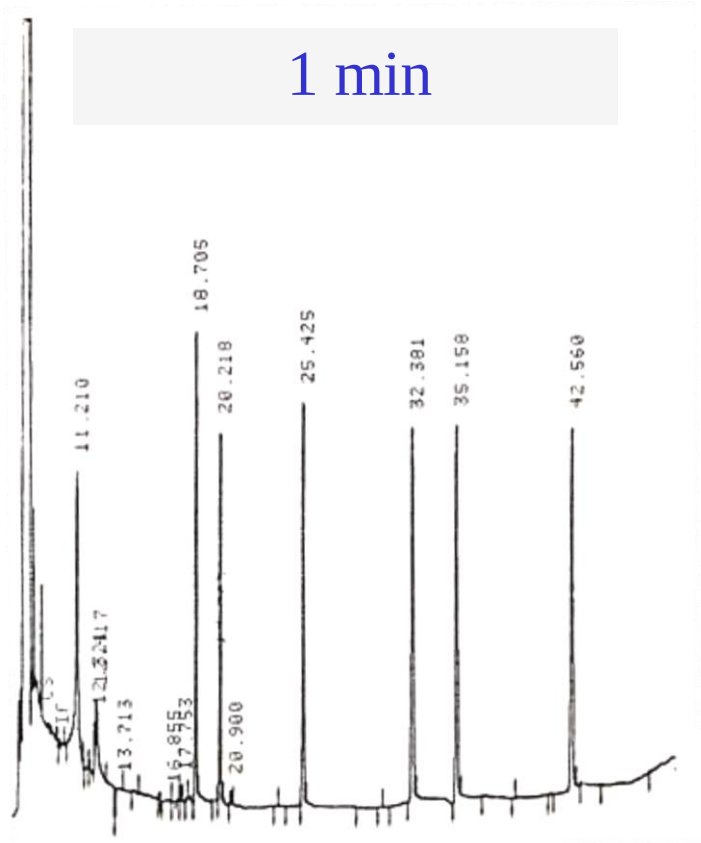
$$t_s = 2 \cdot \frac{V_l}{F}$$

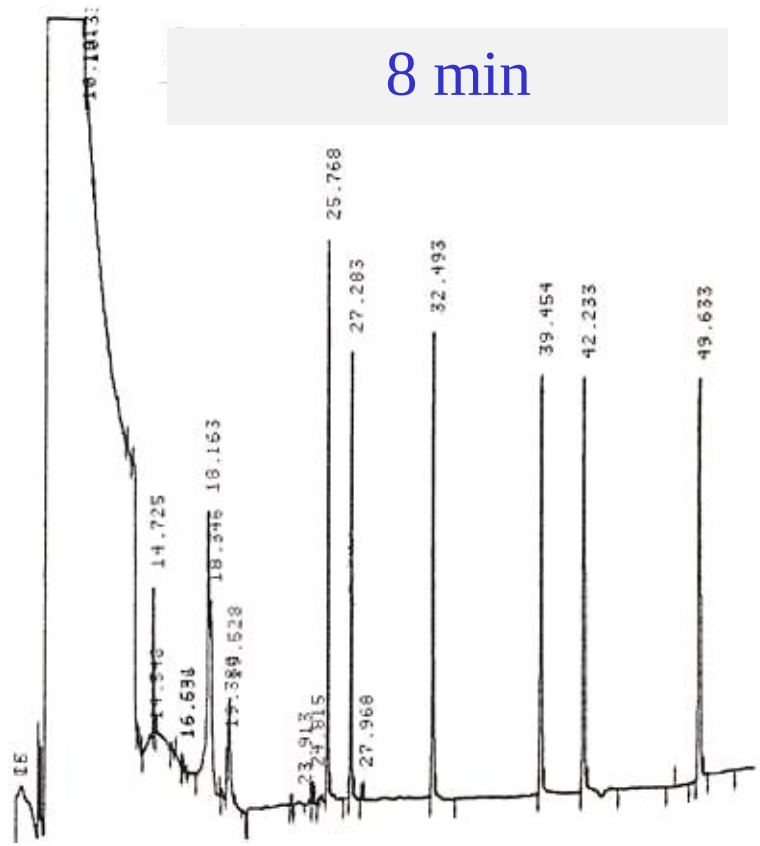
V_l... *liner volume (mL)*
F... *carrier gas flow rate (mL/min)*

Theoretically 1.5 - 2 multiple of time necessary for exchange of carrier gas in injection chamber

INJECTION TECHNIQUES - SPLITLESS

SPLITLESS PERIOD





AREA of PCB 180: **100 %** **116 %**

INJECTION TECHNIQUES - SPLITLESS

BANDBROADENING OF INTRODUCED ZONE:

1. **IN TIME** – slow transfer of sample vapours from inlet to column
2. **IN SPACE** – result of liquid sample migration through column (1 μL = 20 – 30 cm)

FOCUSING OF INTRODUCED ZONE:

IF $\mathbf{k_{front} > k_{rear}} \Rightarrow K_D$ increases, ß decreases

(Distribution ratio $k = K_D / ß$*)*

INJECTION TECHNIQUES - SPLITLESS

FOCUSING OF INTRODUCED ZONE: $k_{front} > k_{rear}$

1) BY STATIONARY PHASE – column must be cooled

2) BY SOLVENT

Column temperature **25-30°C below solvent boiling point** →

condensation - temporary st.ph. with thick film = region with ↓ ß

Analyte capturing (for boiling point similar to solvent)
in narrow band

Temperature programming – consecutive vaporisation

(BANDBROADENING IN SPACE – retention gap)

INJECTION TECHNIQUES - SPLITLESS

FOCUSING OF INTRODUCED ZONE: $k_{front} > k_{rear}$

3) BY TEMPERATURE

Column temperature **min 150°C below boiling point of the most volatile analyte**, solvent is passing through, **analytes condensate**

Temperature programming - consecutive vaporisation – often followed by stationary phase focusing

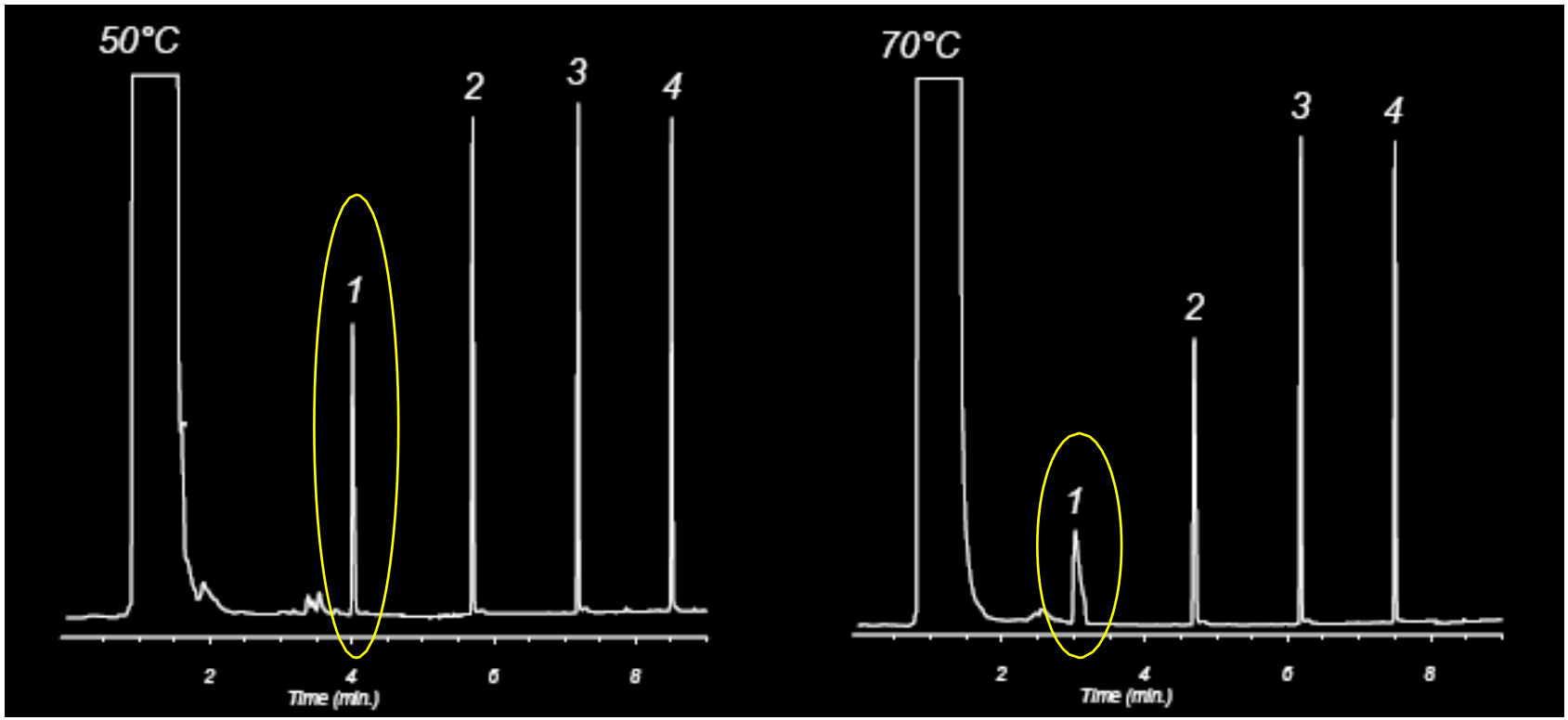
4) USING RETENTION GAP – column without st.ph. ($k \rightarrow 0$)
– minimal retention

Reduction of band length (solvent vaporization)

On column head – focusing by SOLVENT and ST.PH.

INJECTION TECHNIQUES - SPLITLESS

SOLVENT FOCUSING *(hexane,b.p. 68°C)*



(FIGURES: Allen K. Vickers, Agilent Technologies)

INJECTION TECHNIQUES - SPLITLESS

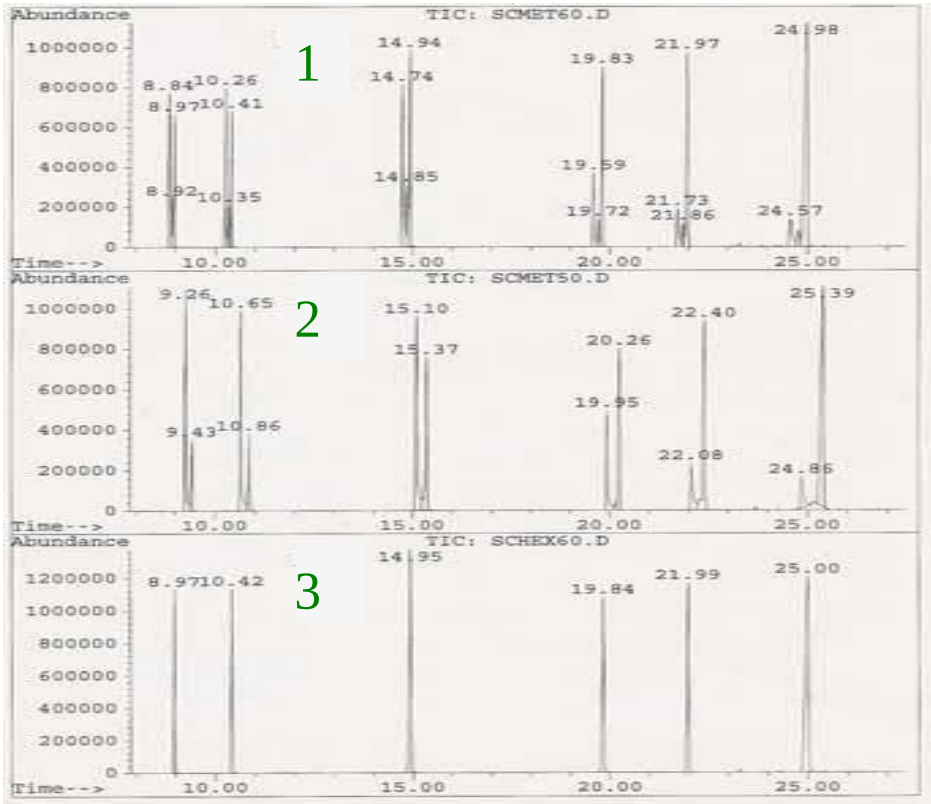
POLARITY of ST.PH. X POLARITY OF SOLVENT

X INITIAL COLUMN TEMPERATURE (DB-5)

1) MeOH, 60°C

2) MeOH, 50°C

3) Hexane, 60°C



GC/MSD – SCAN, phthalates

INJECTION TECHNIQUES - SPLITLESS

OPTIMISATION:

Solvent b.p. min 25°C < b.p. of the most volatile analyte

Initial column temperature 25 – 30°C below solvent b.p.

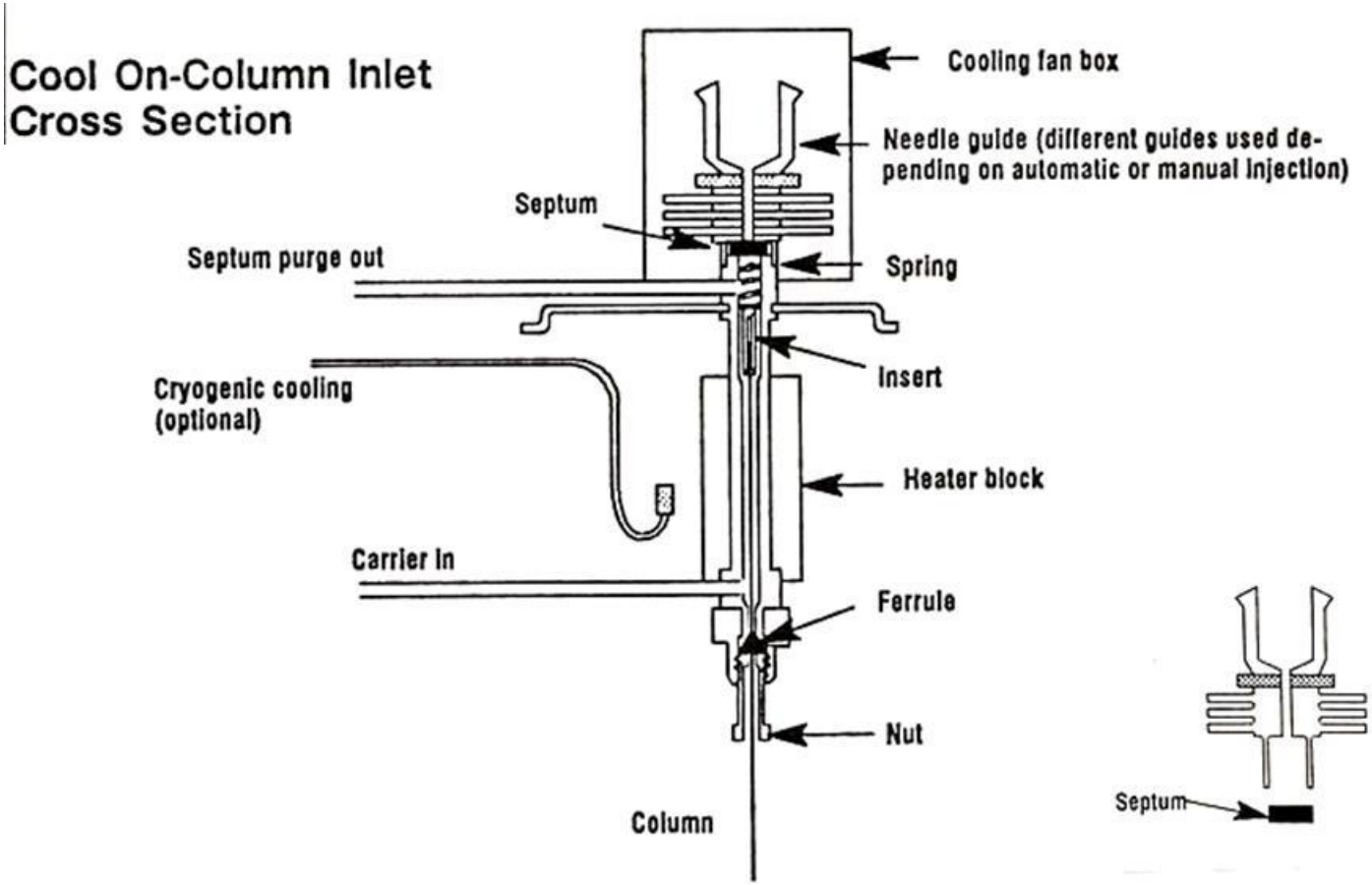
Identical injection volumes

APPLICABILITY:

Diluted samples

Relatively clean samples

INJECTION TECHNIQUES - ON-COLUMN



(Obrázek: Hewlett-Packard (Agilent Technologies))

INJECTION TECHNIQUES - ON-COLUMN

Liquid sample introduced into column – directly without preheating and mixing with carrier gas.

ADVANTAGES:

LOW RISK OF DEGRADATION of analytes during injection

ELIMINATION of DISCRIMINATION

DRAWBACKS:

CONTAMINATION of system by non-volatiles

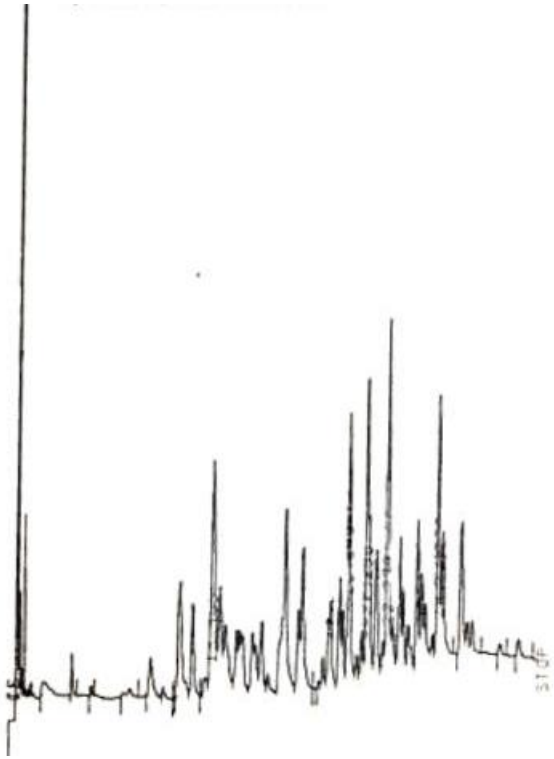
BANDBROADENING of ZONES IN SPACE

RISK of „BACKFLASH“: ↑ column temperature ⇒ vapour pressure > pressure of carrier gas ⇒ expansion in both directions ⇒ wide solvent peak, memory effects

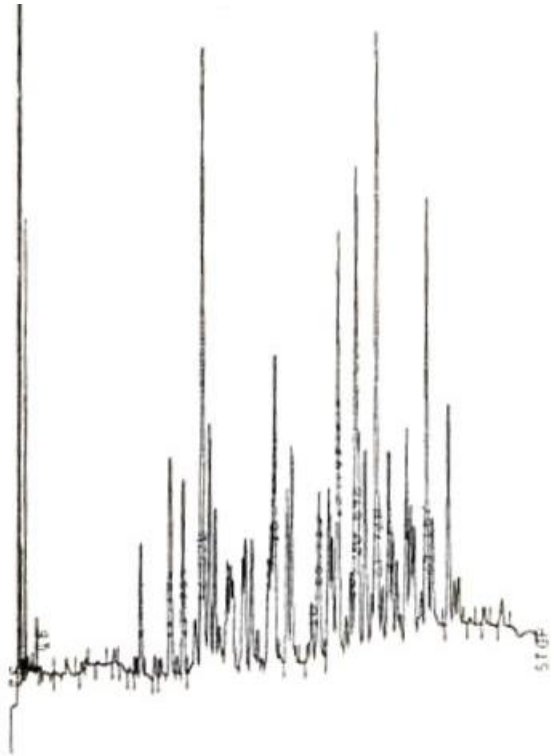
INJECTION TECHNIQUES - ON-COLUMN

SYSTEM CONTAMINATION WITH NON-VOLATILES

Dirty column



1 m of column removed



⇒ **RETENTION GAP** (injection of bigger volumes)

INJECTION TECHNIQUES - ON-COLUMN

DECREASING RISK OF "BACKFLUSH":

Column temperature ≤ solvent boiling point

Fast and continuous injection

Injection of small volumes

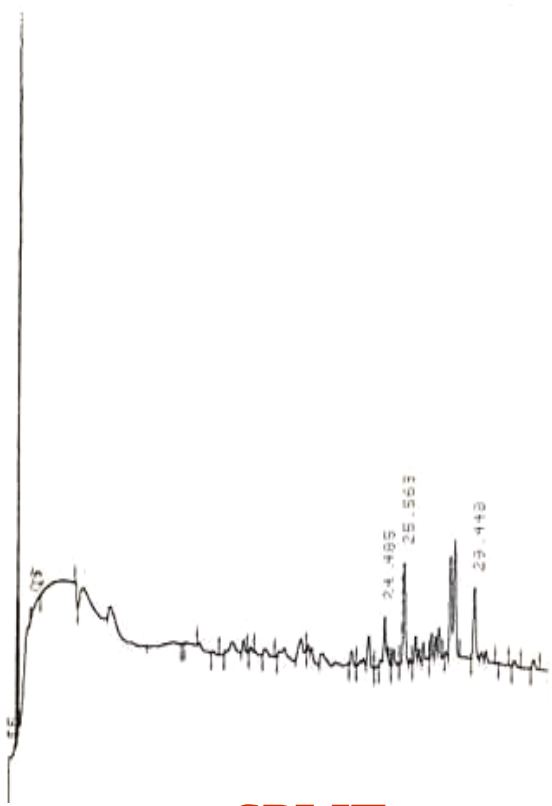
Higher flow rate

Additional cooling of injection chamber

Sharp increase of column temperature after injection

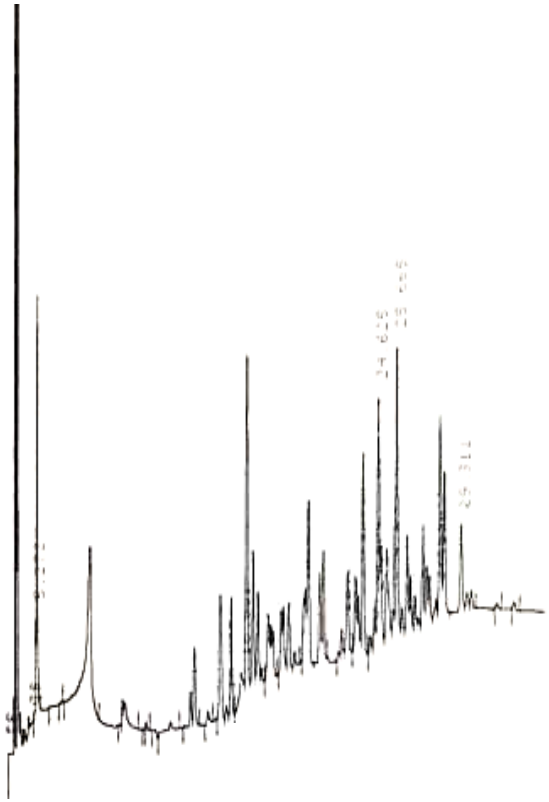
APPLICABILITY - diluted samples, clean samples
- precise results
- before solvent eluted analytes – no focusing
- small injection volumes

INJECTION TECHNIQUES - COMPARISON



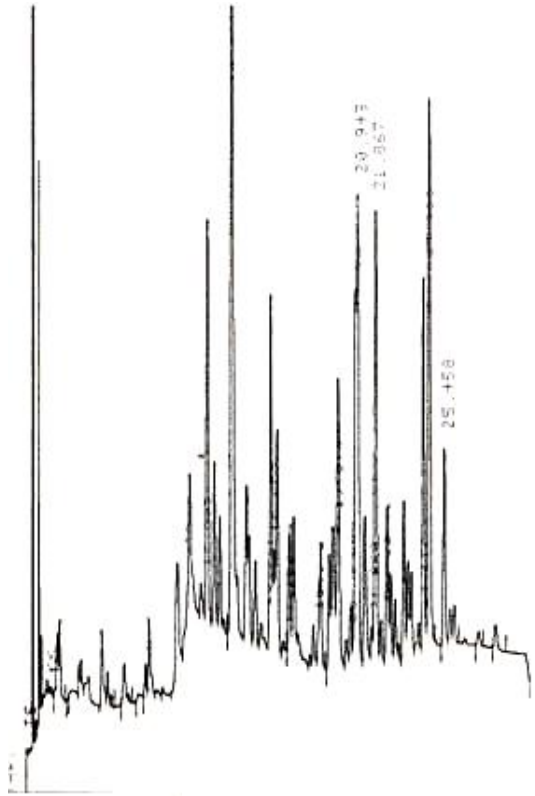
SPLIT

21 %



SPLITLESS

52 %



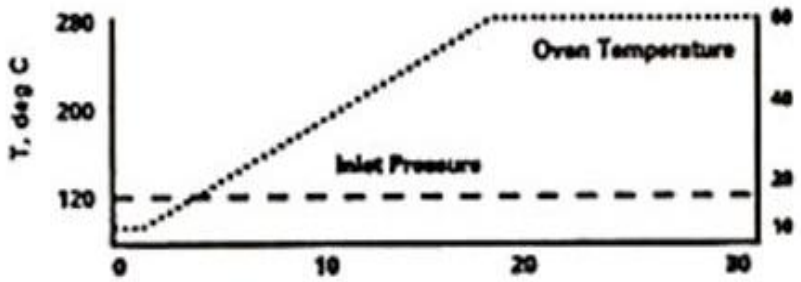
ON-COLUMN

100 %

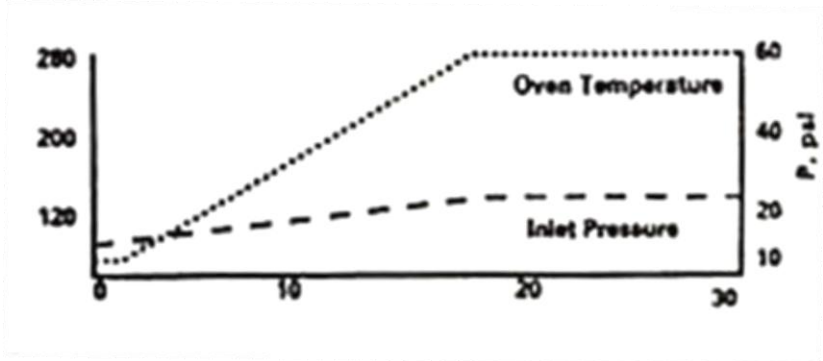
ELECTRONIC PRESSURE CONTROL (EPC)

SPLIT, SPLITLESS, ON-COLUMN, (DETECTOR GASES)

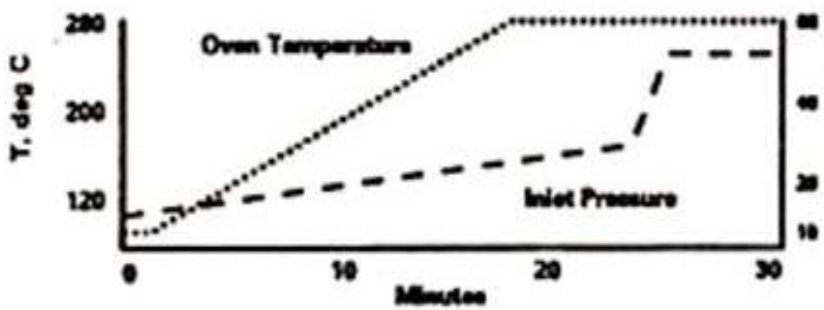
CONSTANT PRESSURE

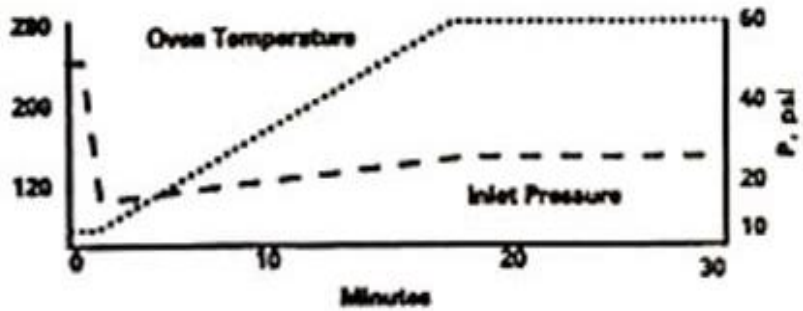


CONSTANT FLOW RATE



PRESSURE PROGRAMMING (pressure pulse followed by const. flow rate





ELECTRONIC PRESSURE CONTROL (EPC)

REASONS FOR EPC APPLICATION – TEMPERATURE PROGRAMMING:

↑ **TEMPERATURE** ⇒↓ RETENTION; ↑ DIFFUSIVITY
⇒↑ OPTIMUM OF LINEAR VELOCITY
FOR CONSTANT EFFICIENCY

X

↑ **TEMPERATURE** ⇒↓ LINEAR VELOCITY
⇒ **PRESURE PROGRAMMING**

EPC ADVANTAGES:

RT reproducibility improvement

Reduction of analysis time

Reduction of discrimination and decomposition of thermolabile compounds

Injection of larger volumes (upto 5 µl)

Resolution improvement (narrower peaks)

Significant especially for shorter and wider columns.

ELECTRONIC PRESSURE CONTROL (EPC)

INJECTION OF LARGER VOLUMES:

INJECTION: 1, 3, 5 µL (= 10, 30, 50 pg PCB per injection)
COLUMN: DB-5 (60 m x 0.25 mm x 0.25 µm)

Relative yield (%) = ($A_{\text{injection n µL}}$ / n * $A_{\text{injection 1 µL}}$) * 100

a) **Constant pressure:** 16 psi = 0.74 mL/min/60°C
0.33 mL/min/270°C

PCB	1 µL	3 µL	5 µL
28	100	93	91
180	100	78	52

b) **Constant flow rate:** 0.74 mL/min = 16 psi/60°C
28.2 psi/270°C

PCB	1 µL	3 µL	5 µL
28	100	85	94
180	100	89	118

ELECTRONIC PRESSURE CONTROL (EPC)

INJECTION OF LARGER VOLUMES:

c) Pressure pulse: 150, 200, 250 kPa during splitless period, followed by constant flow rate 0.74 mL/min

150 kPa

PCB	1 µL	3 µL	5 µL
28	100	89	91
180	100	85	94

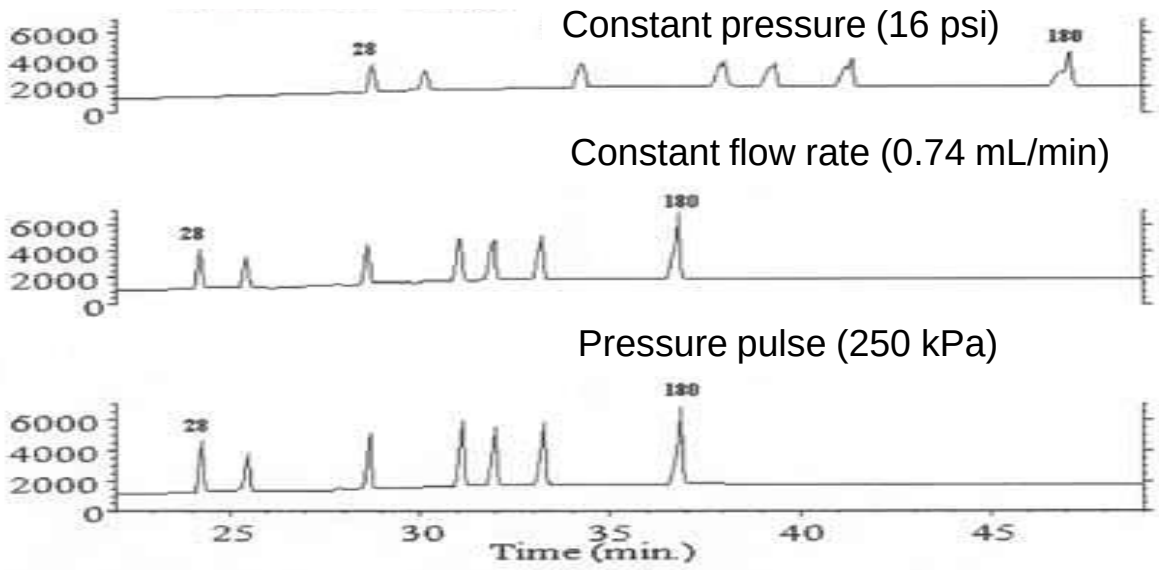
200 kPa

1 µL	3 µL	5 µL
100	88	95
100	99	100

250 kPa

1 µL	3 µL	5 µL
100	97	97
100	96	100

Injection 5 µL = 50 pg



LARGE VOLUME INJECTION (LVI)

REASONS:

- **LOD** 1 μL - 1 ppm
  ⬇
  50 μL 20 ppb
  ⬇
  50 x lower LOD

- **SIMPLIER SAMPLE PREPARATION**
  - concentration step is not necessary
  - possibility of decreasing of a sample weight

- **ON-LINE APPLICATIONS**
  - sample preparation - GC a GC/MS with SPE
  - HPLC with GC

LARGE VOLUME INJECTION (LVI)

PROBLEMS:

Large amount of solvent

X

Columns, detectors (MSD)

Bandbroadening

SOLUTIONS:

Solvent removal before analytical column

Focusing techniques

REALISATION:

- COOL ON-COLUMN INJECTION (**COC**)
- COC with solvent vapour exit (**COC-SVE**)
- TEMPERATURE PROGRAMMED SPLIT/SPLITLESS INJECTION (Programmed Temperature Vaporizing Injector, **PTV**)

LARGE VOLUME INJECTION (LVI)

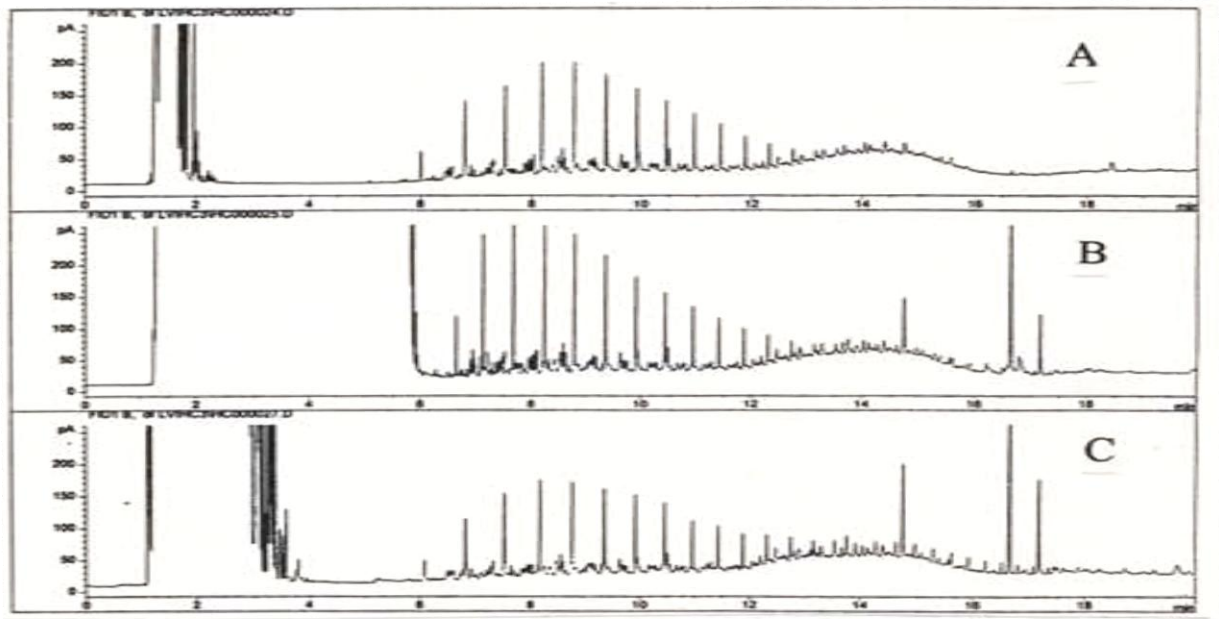
TECHNIQUE	DEMANDS	INJECTION	LIMITATION
COC	• 5 - 10m pre column	upto 100 µL	non-volatiles accumulation in column
COC-SVE	• pre column • S V E	upto 1 ml	non-volatiles accumulation in column
PTV	• controlled injection speed • packed liner (•cryo-cooling)	upto 1 ml	loss of volatiles

LARGE VOLUME INJECTION (LVI)

COC, COC-SVE

Reference sample of mineral oil in hexane, GC/FID:

A) concentration 1 mg/mL, injection **1µL, COC**
B) diluted 50x (=0.02 mg/mL), injection **50 µL, COC-precolumn**
C) diluted 50x (=0.02 mg/ml), injection **50 µL, COC-SVE**



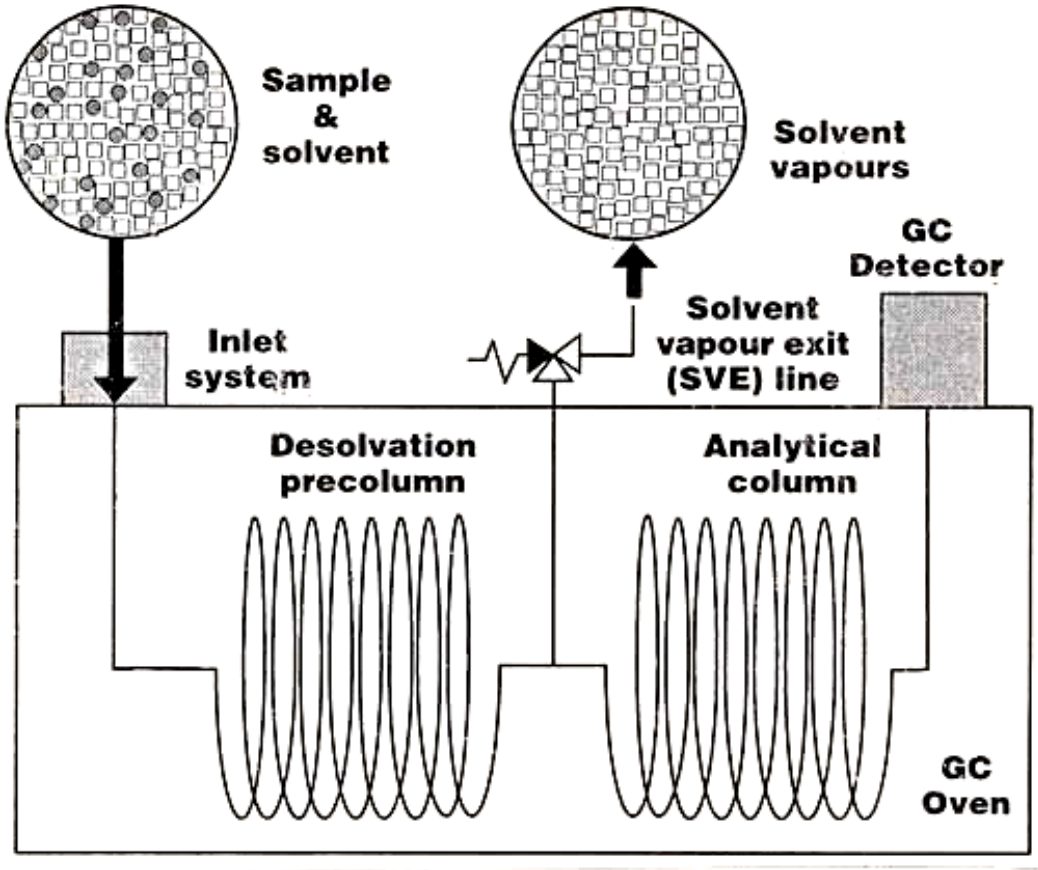
(F.David et al.: poster - 18. ISCC, 20.-24.5.1996, Riva del Garda, Italy)

LARGE VOLUME INJECTION (LVI)

COC-SVE

Solvent removal behind the precolumn

Programming of column temperature

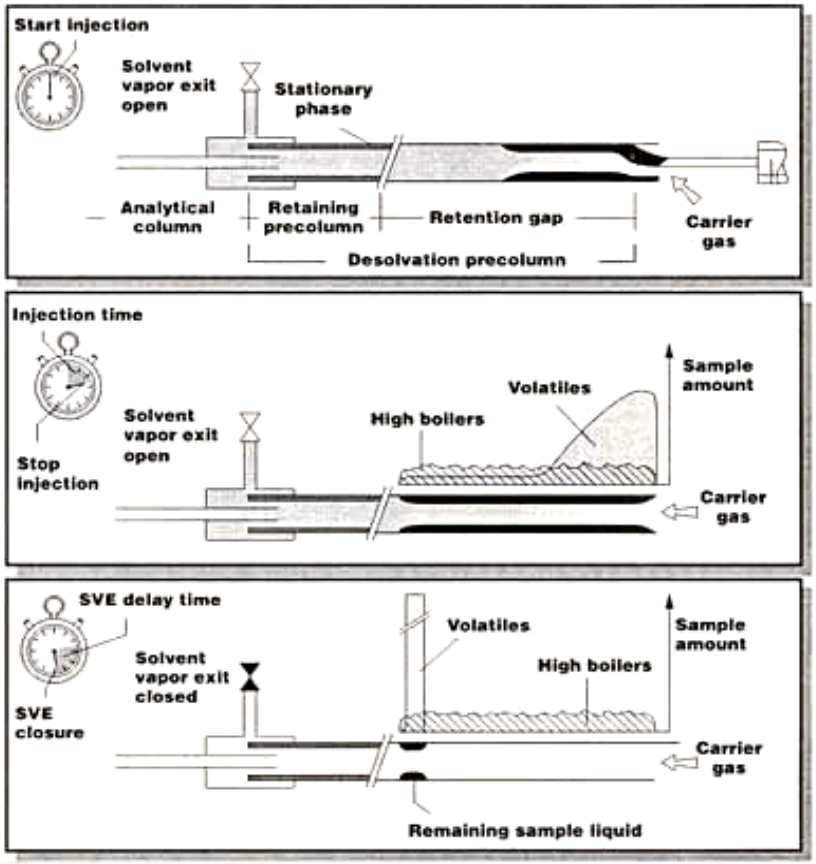


LARGE VOLUME INJECTION (LVI)

COC-SVE

Minimal losses of volatiles

For „clean“ samples (contaminated pre column causes peak tailing)

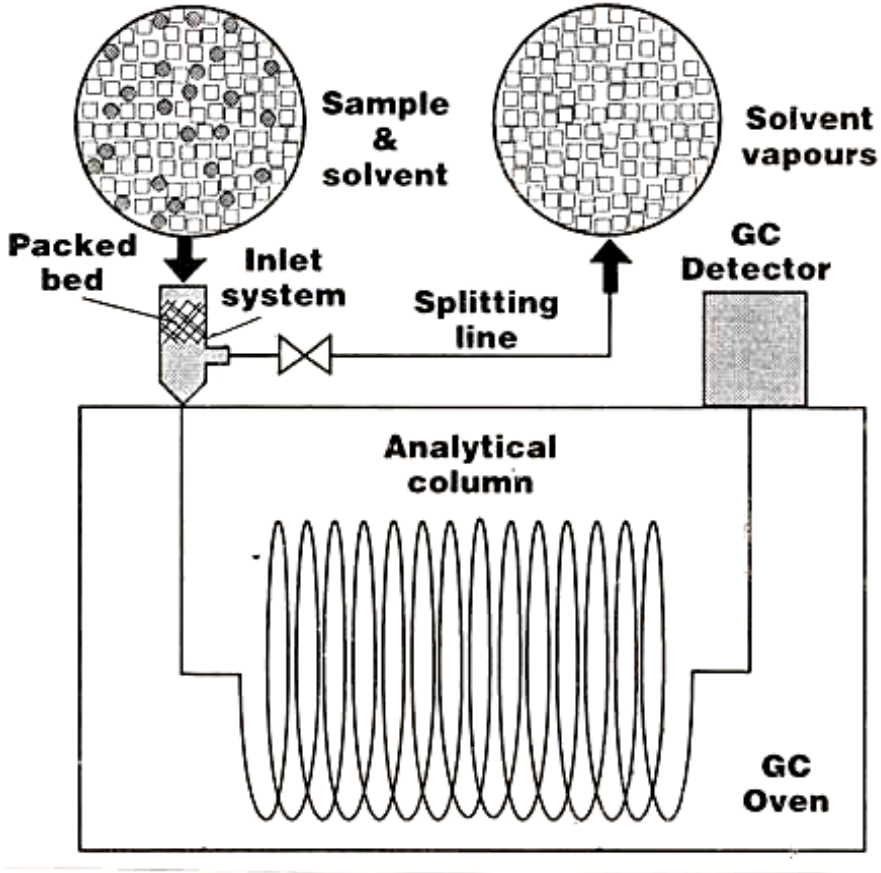


LARGE VOLUME INJECTION (LVI)

PTV

Solvent removal in injection chamber

Programming of injection chamber temperature



LARGE VOLUME INJECTION (LVI)

PTV

DEMANDS:

Small volume of injection chamber → faster heating

Small volume of liner → faster heating, approx. 15 - 150 μL

Liner packed with glass wool or various support (liquid sample retention)

IF injection speed = solvent evaporation speed ⇒ theoretically, it is possible to inject unlimited amount of sample

1) Solvent sensor – split line, provides information about solvent evaporation speed - set up of amount intended for removal
2) Injection speed is regulated

LARGE VOLUME INJECTION (LVI)

PTV

SPLIT – SPLITLESS INJECTION (solvent split injection)

- Sample injected to cool injection chamber with open split
- **Solvent and volatiles are transferred to split**
- **After split is off, injection chamber is heated, analytes are introduced into column**

Advantages / limitations:

- Loss of volatiles (optimisation of liner packaging, solvent effect)
- Less problems with column contamination
- No discrimination in needle and no degradation of thermolabile compounds (compare to classic split/splitless)

LARGE VOLUME INJECTION (LVI)

COC x PTV