INJECTION TECHNIQUES IN GC
≈ *CAPILLARY COLUMNS* ≈

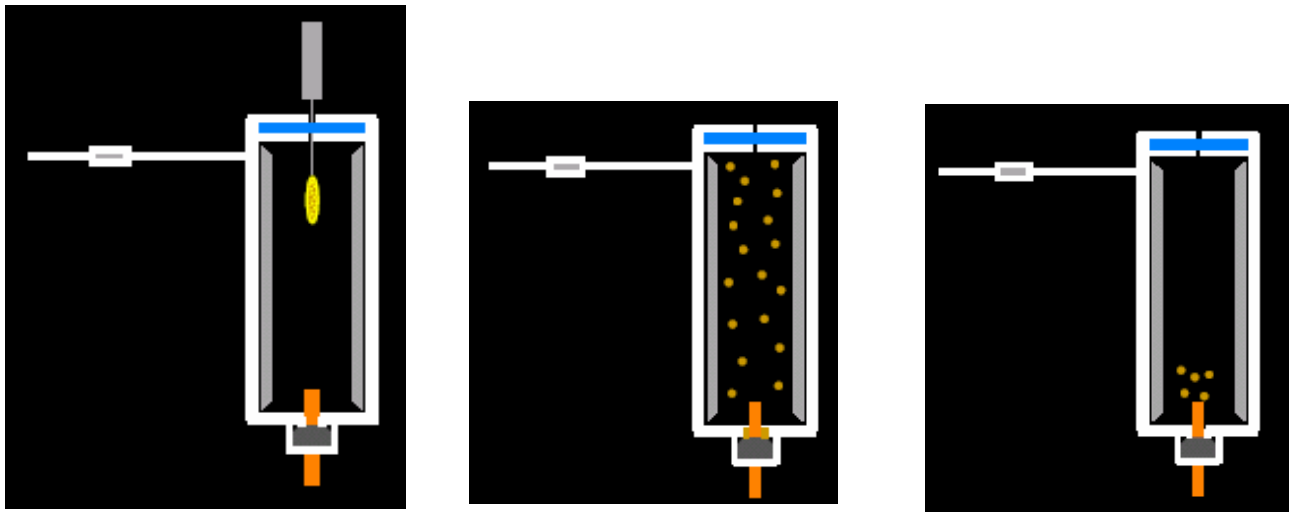
FLASH VAPORISATION INJECTION

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

COOL INJECTION – Large Volume Injection (LVI)

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

FLASH VAPORISATION INJECTION TECHNIQUES I



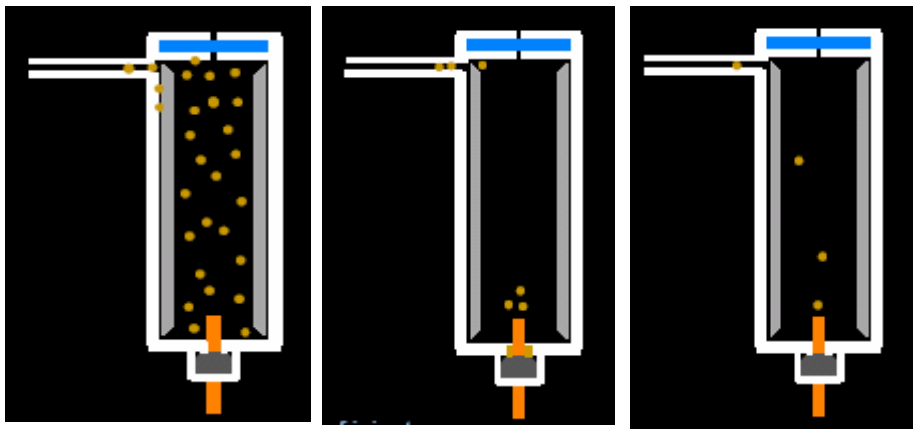
(FIGURES: Allen K. Vickers, Agilent Technologies)

RISKS: BACKFLASH and DISCRIMINATION

FLASH VAPORISATION INJECTION TECHNIQUES II

BACKFLASH

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



(FIGURES: Allen K. Vickers, Agilent Technologies)

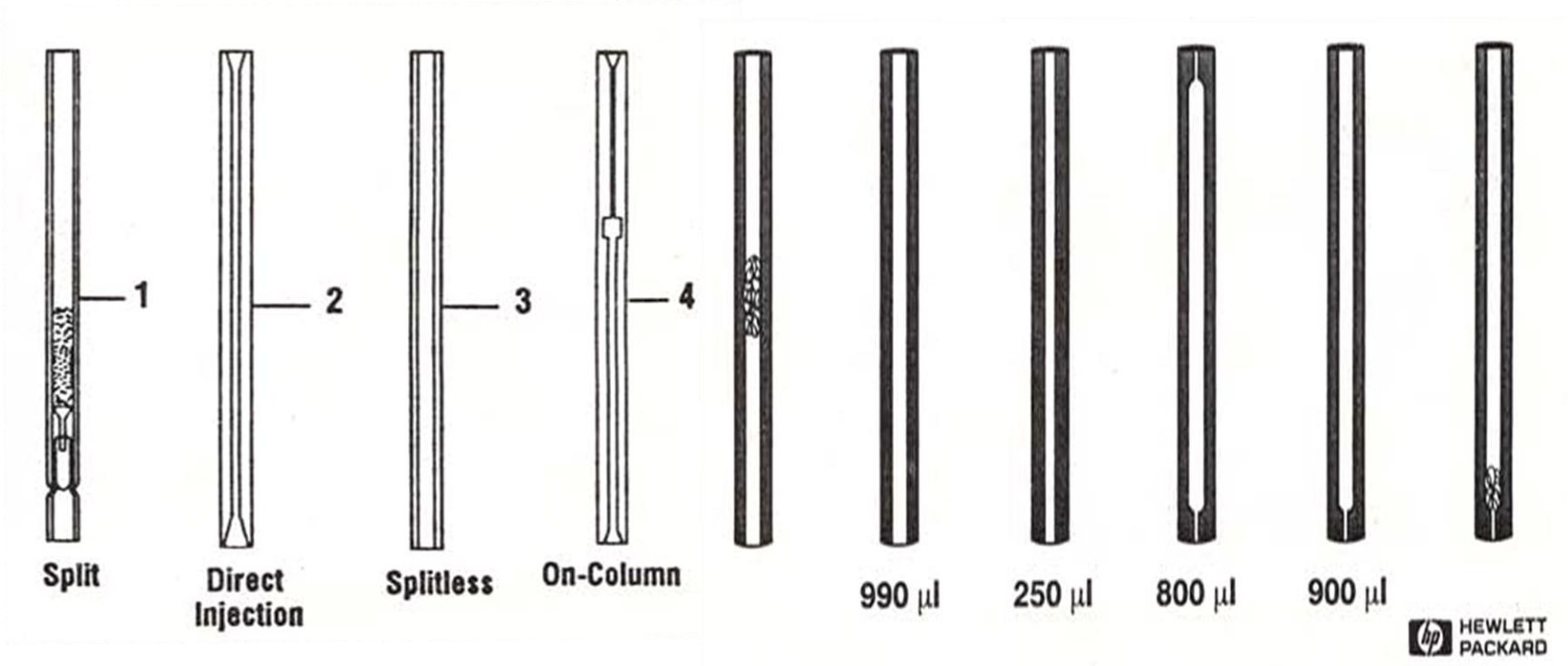
- samples losses
- tailing solvent
- ghost peaks

Minimization:

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

FLASH VAPORISATION INJECTION TECHNIQUES III

Used liners (inserts, inserts for injector)
- define the internal volume and behavior of the sample

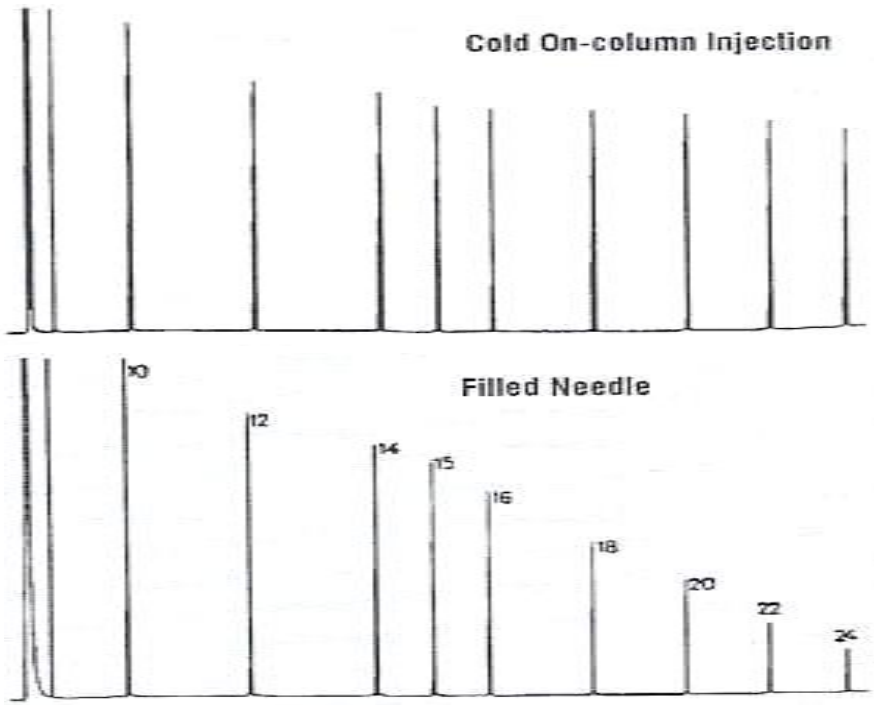


(FIGURE:Hewlett-Packard (Agilent Technologies))

FLASH VAPORISATION INJECTION TECHNIQUES IV

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**

FLASH VAPORISATION INJECTION TECHNIQUES V

MANUAL INJECTION

FILLED NEEDLE

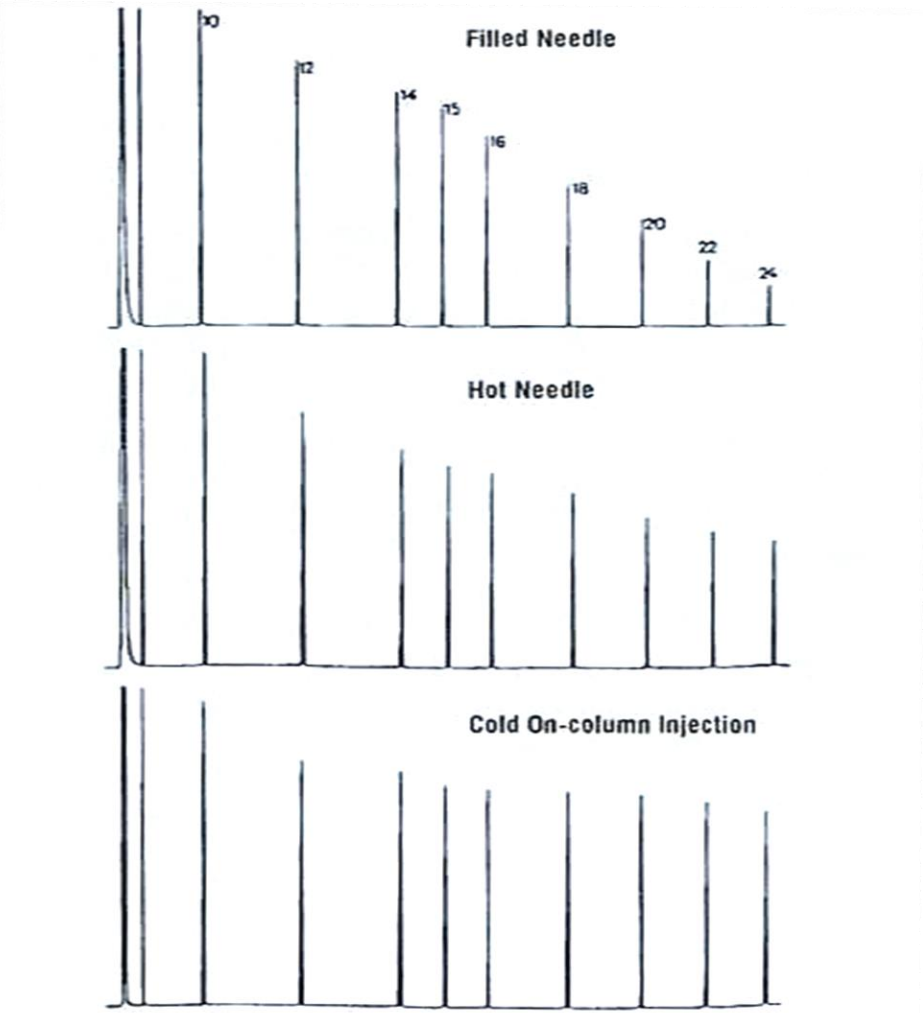
COLD NEEDLE

HOT NEEDLE *

SOLVENT FLUSH *

AIR FLUSH *

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



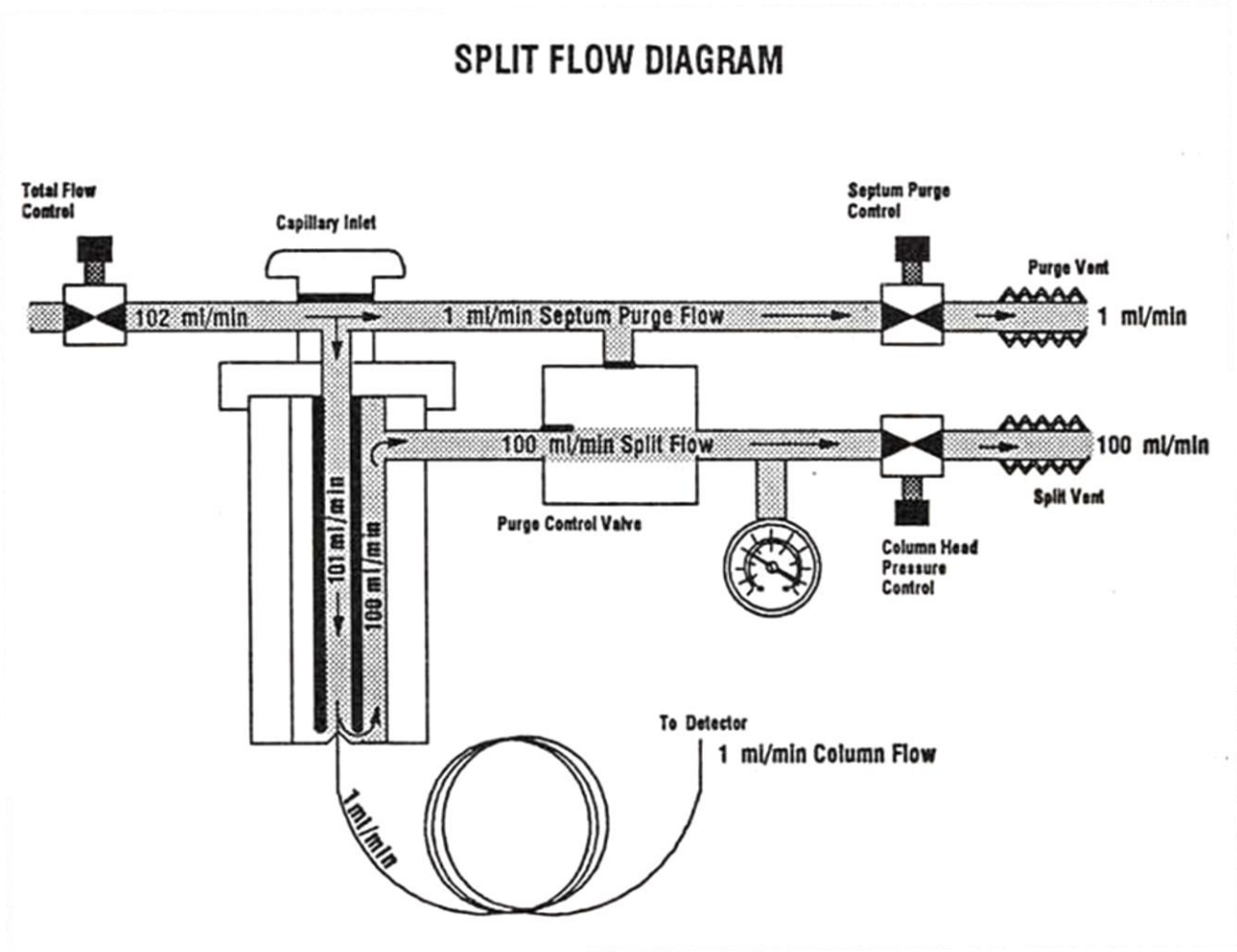
(FIGURES: Allen K. Vickers, Agilent Technologies)

FLASH VAPORISATION INJECTION TECHNIQUES VI

MANUAL xxx AUTOMATIC INJECTION (hot needle)

	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 PARAMETERS - SPLIT I



(FIGURE:Hewlett-Packard (Agilent Technologies))

INJECTION TECHNIQUES PARAMETERS - SPLIT II

SPLIT = injection technique with carrier gas flow split

We set the split ratio between the column and the gas outlet

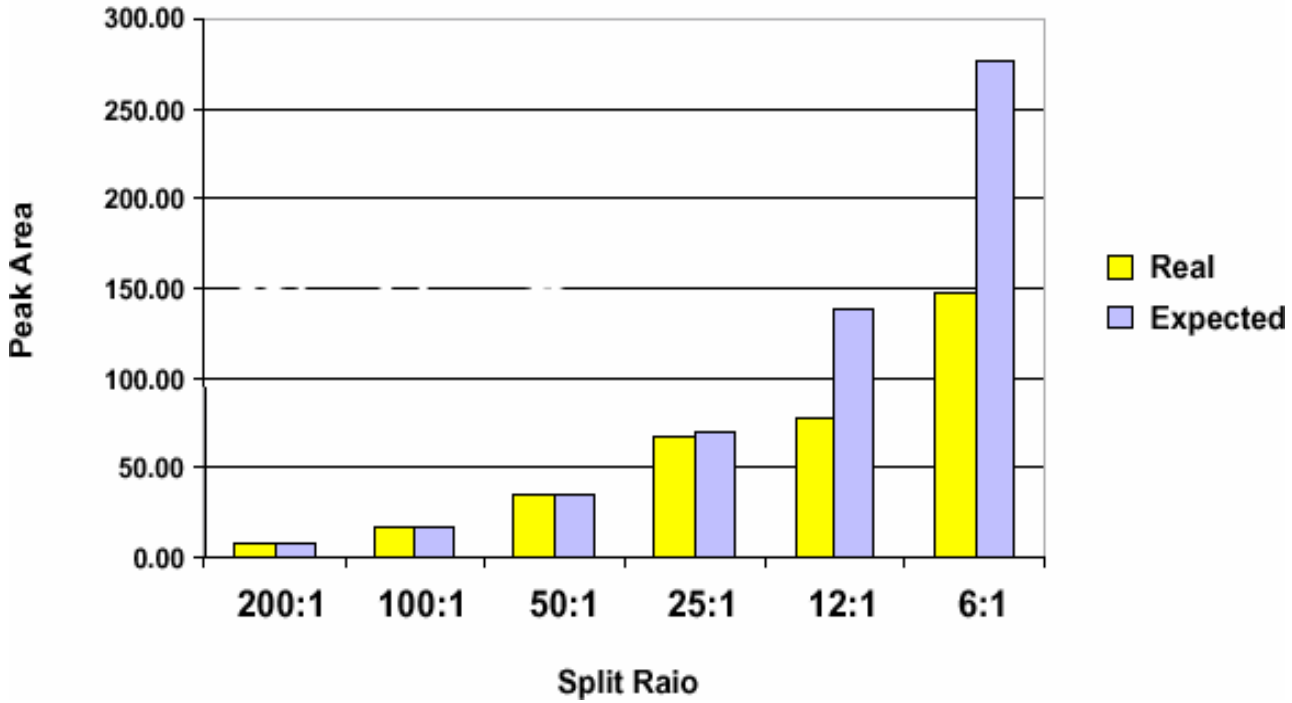
Split ratio suitable with respect to i.d. columns and sample concentrations

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

INJECTION TECHNIQUES PARAMETERS - SPLIT III

Split ratio determines an amount introduced into column

Split ratio ≠ sample partitioning ratio



(FIGURE: SGE, www.sge.com)

SPLIT RATIO

Ideal case

- sample completely in gas phase, homogeneously mixed with carrier gas

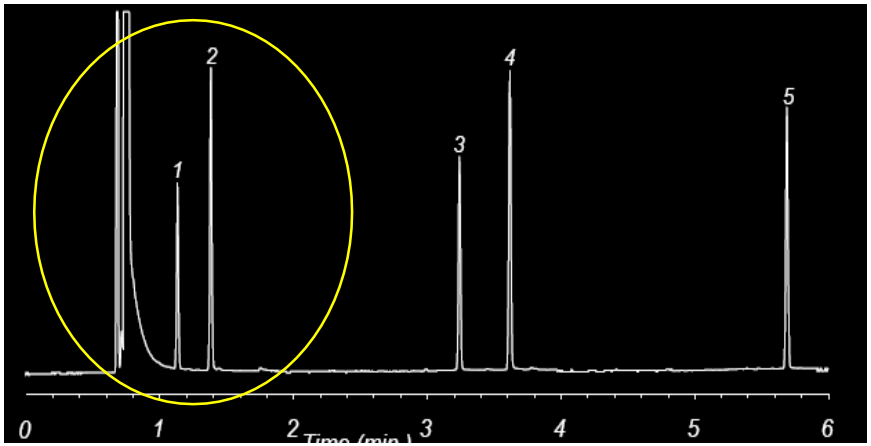
Real case

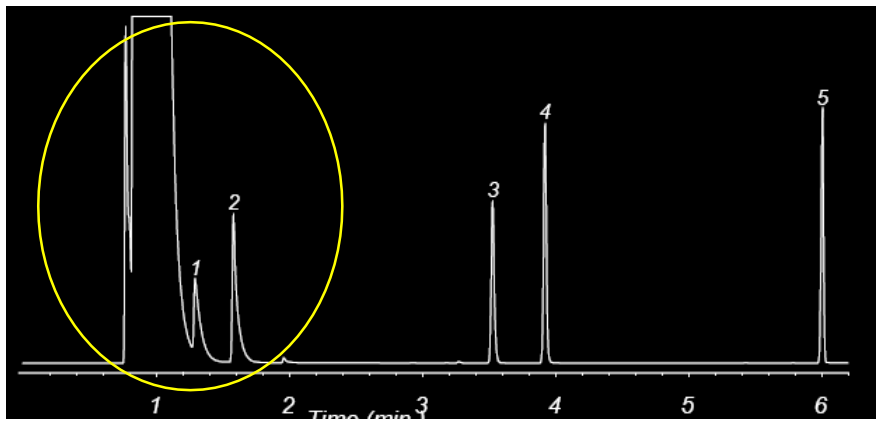
- sample contains components with different volatility
- incomplete evaporation
- various diffusivity of sample components
- fluctuating split ratio

⇒ DISCRIMINATION (distorted composition)

⇒ WORSE REPEATABILITY

INJECTION TECHNIQUES PARAMETERS - SPLIT V





DB-1 (15m x 0.25mm x 0.25µm)

(FIGURE: Hewlett-Packard (Agilent Technologies))

SPLIT RATIO

1 : 200

1 : 5

INJECTION TECHNIQUES PARAMETERS - SPLIT VI

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 PARAMETERS - SPLIT VII

REDUCTION 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

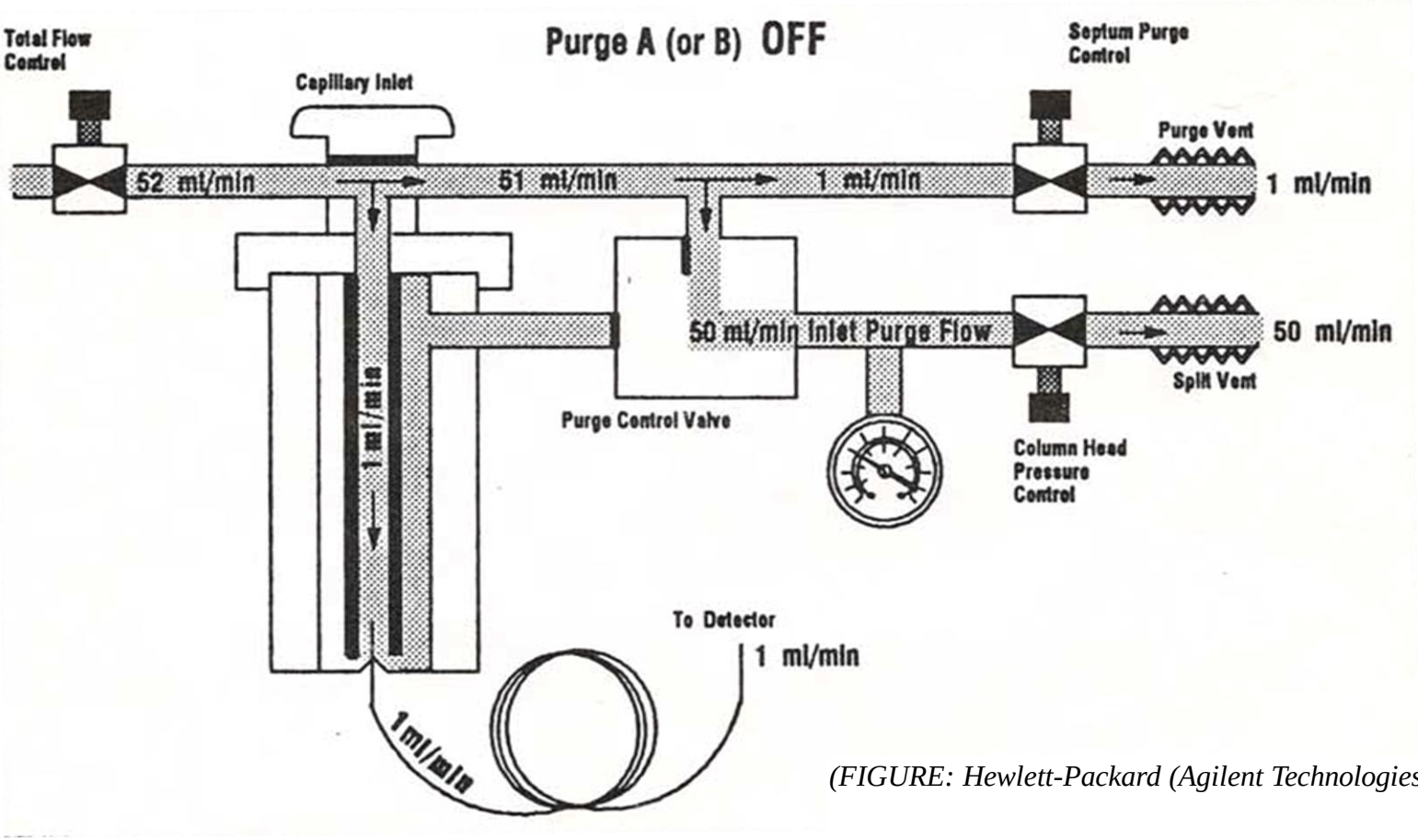
Dirty samples

High concentration of analytes

For columns with very small i.d.

INJECTION TECHNIQUES PARAMETERS - SPLITLESS I

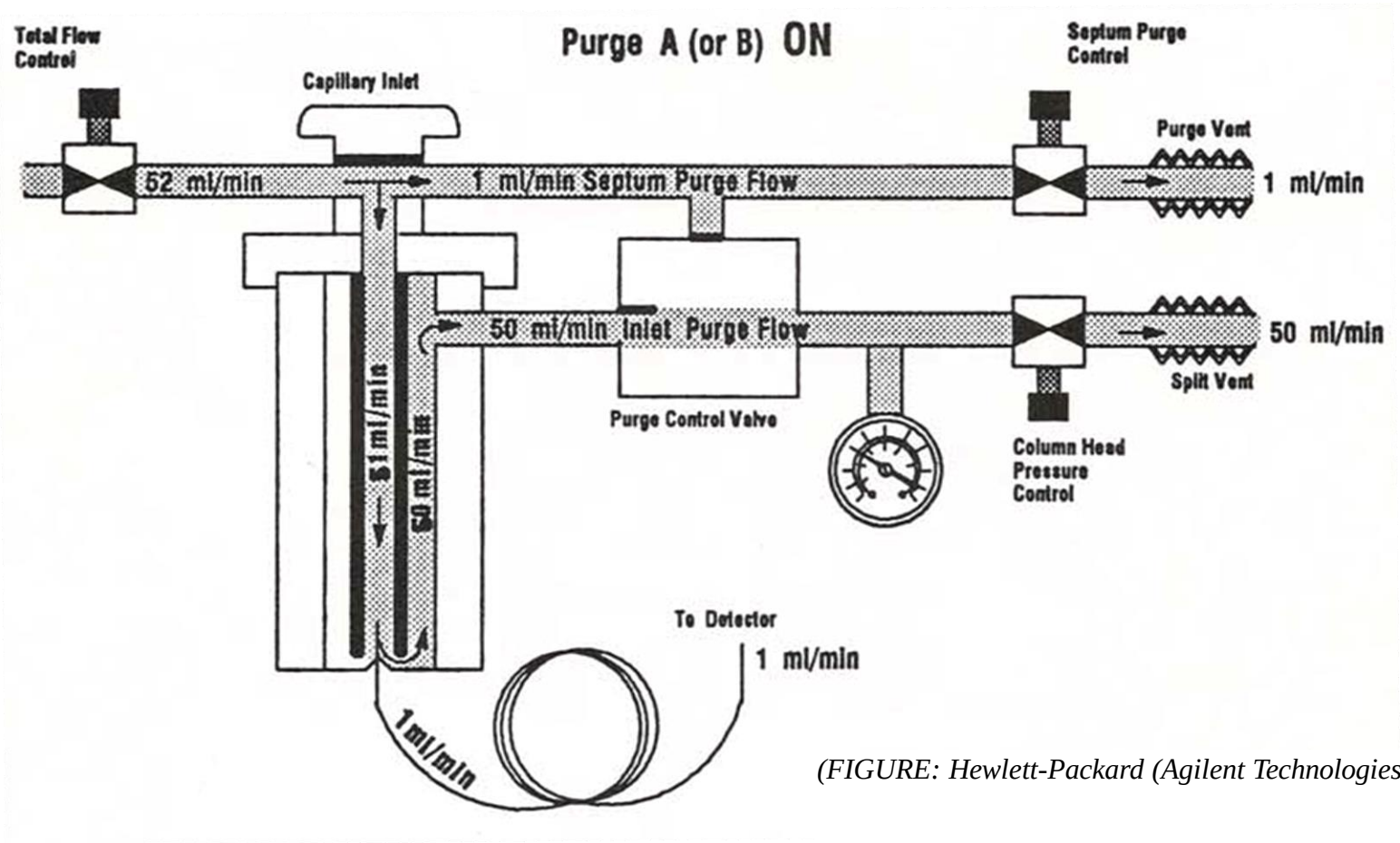
SAMPLE INTRODUCTION INTO COLUMN – splitless period (STEP 1)



(FIGURE: Hewlett-Packard (Agilent Technologies))

INJECTION TECHNIQUES PARAMETERS - SPLITLESS II

SAMPLE INTRODUCTION INTO COLUMN – split period (STEP 2)



(FIGURE: Hewlett-Packard (Agilent Technologies))

INJECTION TECHNIQUES PARAMETERS - SPLITLESS III

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

Solvent properties

Analytes properties

Injection chamber volume

Injected volume

Injection speed

Carrier gas flow rate

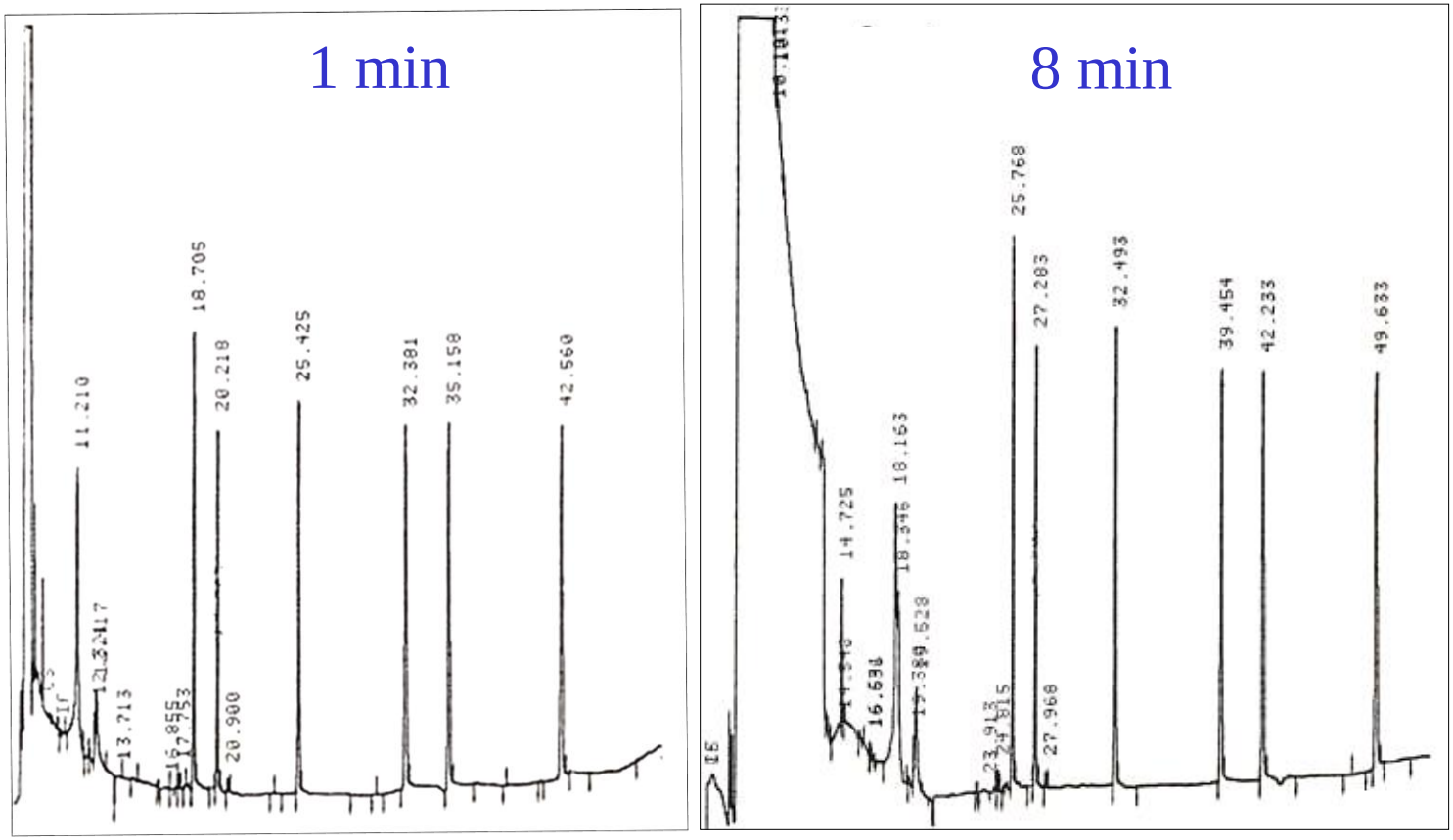
$$t_s = 2 \cdot \frac{V_l}{F}$$

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 PARAMETERS - SPLITLESS IV

Different splitless period - 1 vs. 8 min
16% increase in response, wide solvent zone



INJECTION TECHNIQUES PARAMETERS - SPLITLESS V

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 $k = K_D / ß$*)*

FOCUSING OF INTRODUCED ZONE

1. By means of SP - column must be cooled

2. By means of solvent - column temperature 25-30 °C below solvent b.p.t.; condensation occurs - a temporary secondary SP with a thick film is formed = region with ↓ß (= $r/2.d_f$)

- analyte capturing in narrow band (for b.p.t. similar to solvent)

- subsequent temperature programming - gradual evaporation

FOCUSING OF INTRODUCED ZONE

3. By means of temperature - column temperature min. 150 °C below b.p.t. of the most volatile analyte, solvent is passing through, analytes condensate

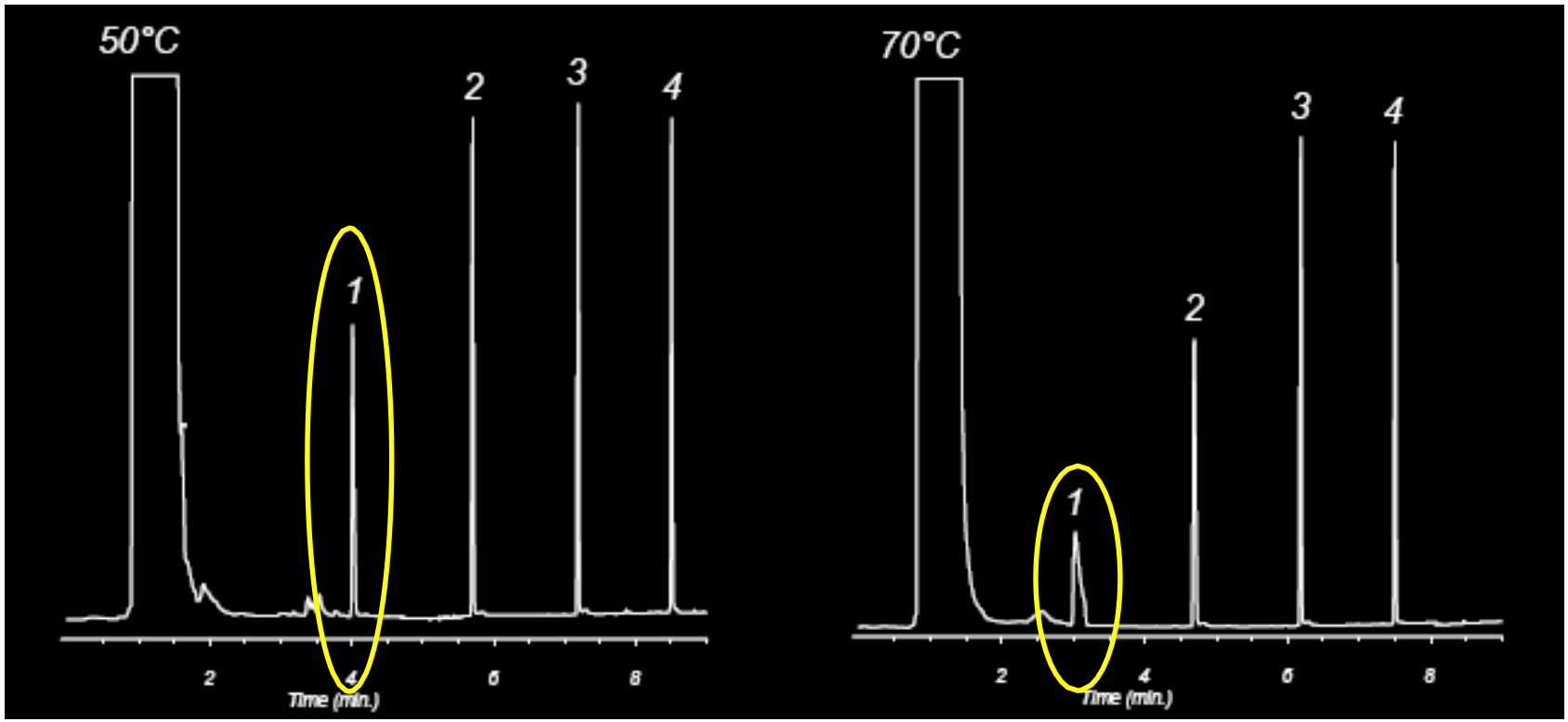
- subsequent temperature programming - gradual evaporation often followed by SP focusing

4. By means of retention gap - column without SP ($k \rightarrow 0$)

- min. retention
- reduction of band length (solvent vaporization)
- on column head – focusing by SOLVENT and SP

INJECTION TECHNIQUES PARAMETERS - SPLITLESS VIII

FOCUSING OF INTRODUCED ZONE BY MEANS OF SOLVENT



(n-hexane, b.p.t. 69 °C)

(FIGURES: Allen K. Vickers, Agilent Technologies)

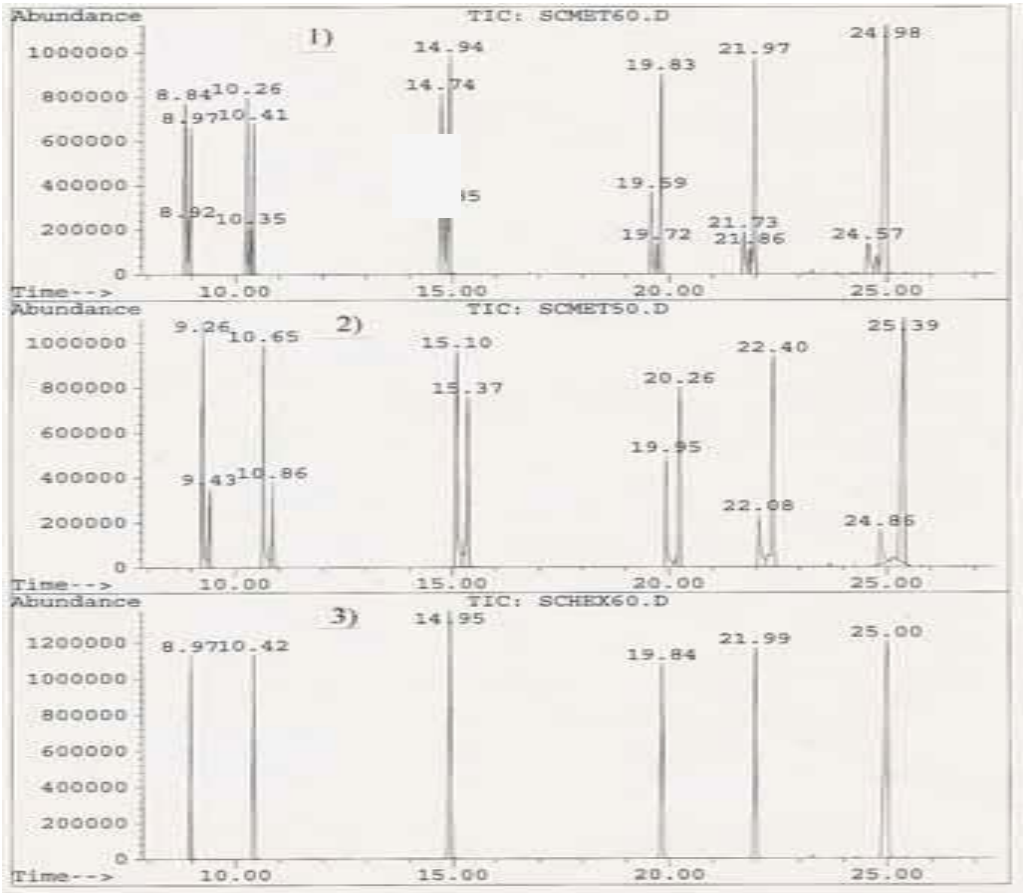
INJECTION TECHNIQUES PARAMETERS - SPLITLESS IX

POLARITY OF SP VS. *POLARITY OF SOLVENT*
VS. *INITIAL COLUMN TEMPERATURE* (DB-5, GC/MSD, PHTHALATES)

1) MeOH, 60 °C

2) MeOH, 50 °C

3) n-Hexane, 60 °C



INJECTION TECHNIQUES PARAMETERS - SPLITLESS X

OPTIMISATION:

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

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

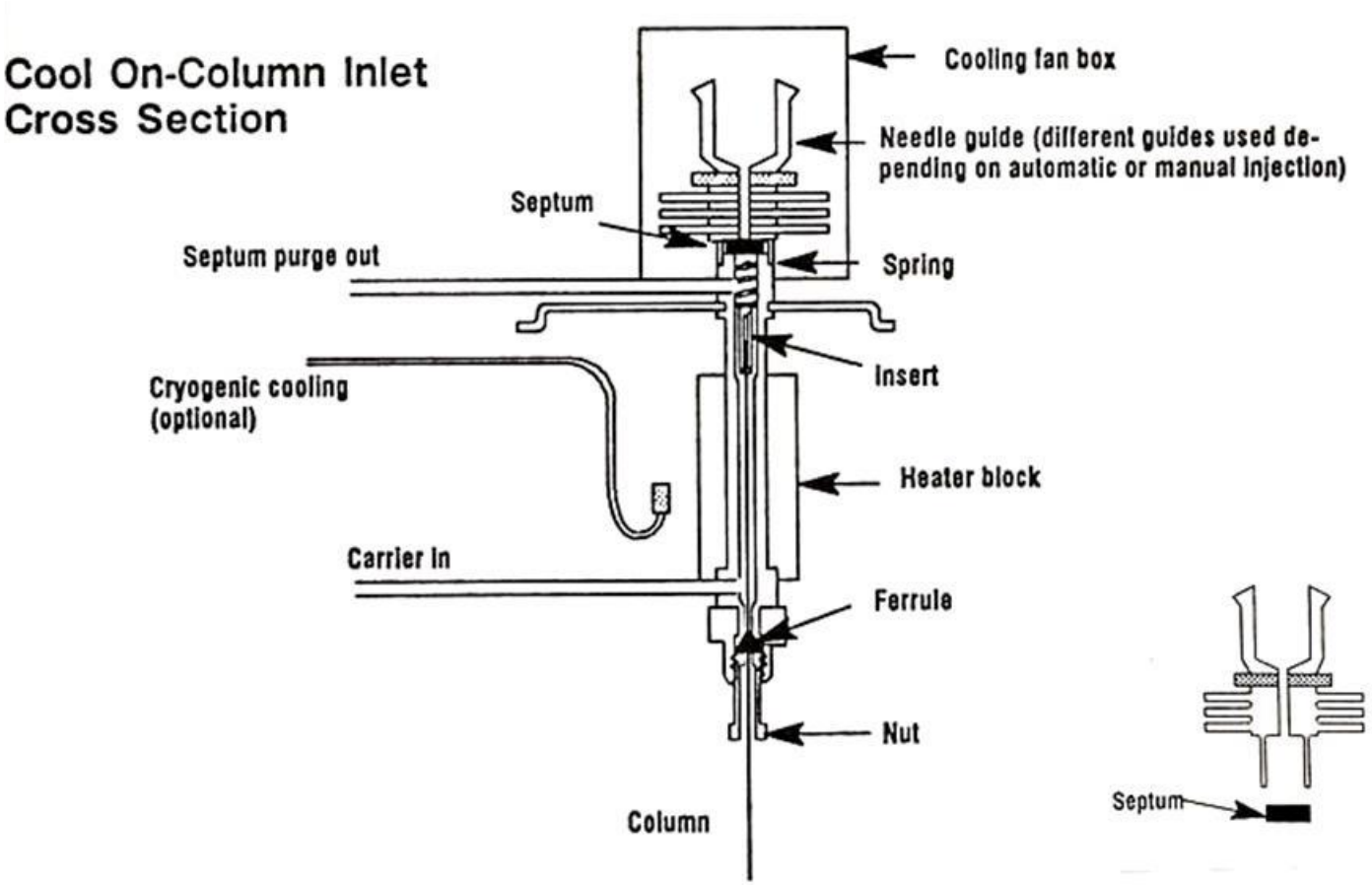
Identical injection volumes

APPLICABILITY:

Diluted samples (concentration and number o analytes)

Relatively clean samples (matrix components)

INJECTION TECHNIQUES PARAMETERS – ON-COLUMN I



(FIGURE: Hewlett-Packard (Agilent Technologies))

INJECTION TECHNIQUES PARAMETERS – ON-COLUMN II

Liquid sample introduced into column – directly without preheating and mixing with carrier gas (syringe with tapered needle at the end).

ADVANTAGES

- low risk of degradation of analytes during injection

- elimination of discrimination

DRAWBACKS

- contamination of system by non-volatiles

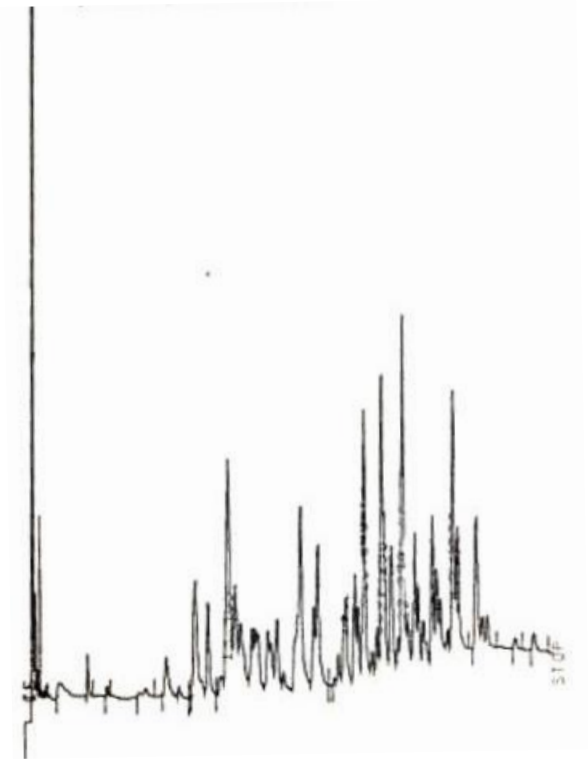
- bandbroadening of zones in space

- risk of „backflash“ - ↑ column temperature
⇒ vapour pressure > pressure of carrier gas ⇒ expansion in both directions ⇒ wide solvent peak, risk of memory effects

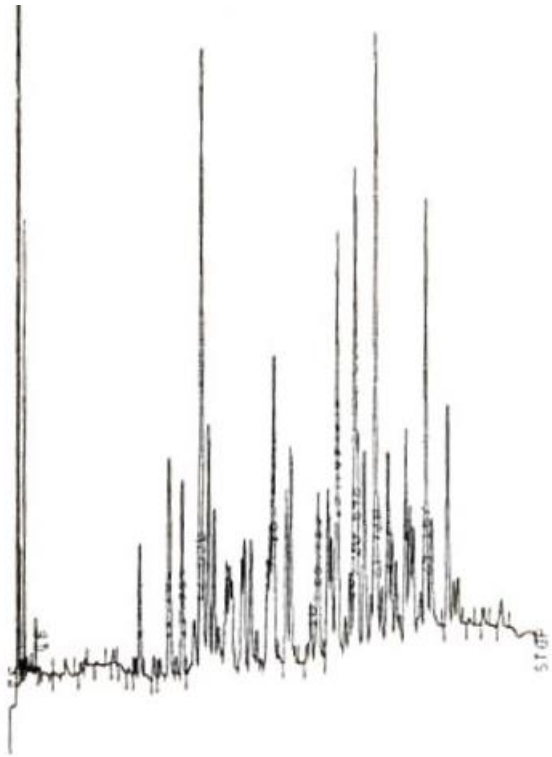
INJECTION TECHNIQUES PARAMETERS – ON-COLUMN III

DEPOSITION OF NON-VOLATILES IN THE COLUMN

Dirty column



Column after removal of 1 m from the injection side



⇒ **RETENTION GAP** (possibility of larger volumes injection)

INJECTION TECHNIQUES PARAMETERS – ON-COLUMN IV

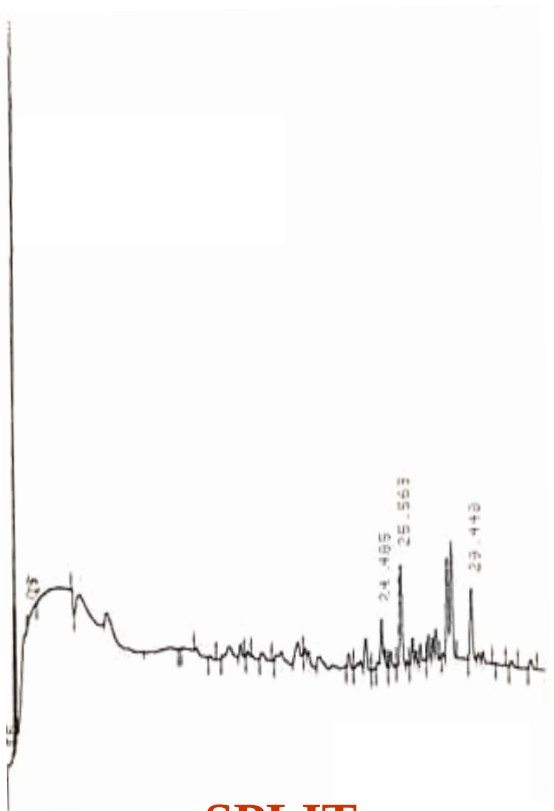
BACKFLUSH RISK REDUCTION

- **column temperature ≤ solvent boiling point**
- **combination of cooling and pulse injection**
- fast and continuous injection
- injection of small volumes
- higher flow rate
- additional cooling of injection chamber
- sharp increase of column temperature after injection

APPLICABILITY

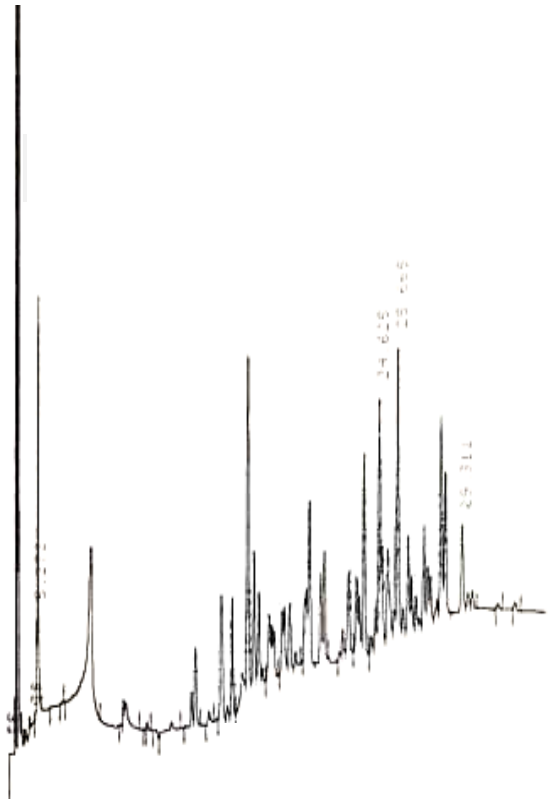
- diluted and clean samples
- analytes eluting before solvent cannot be focused
- **high response = low LOD**

INJECTION TECHNIQUES COMPARISON: SAMPLE AMOUNT TRANSFERRED TO COLUMN



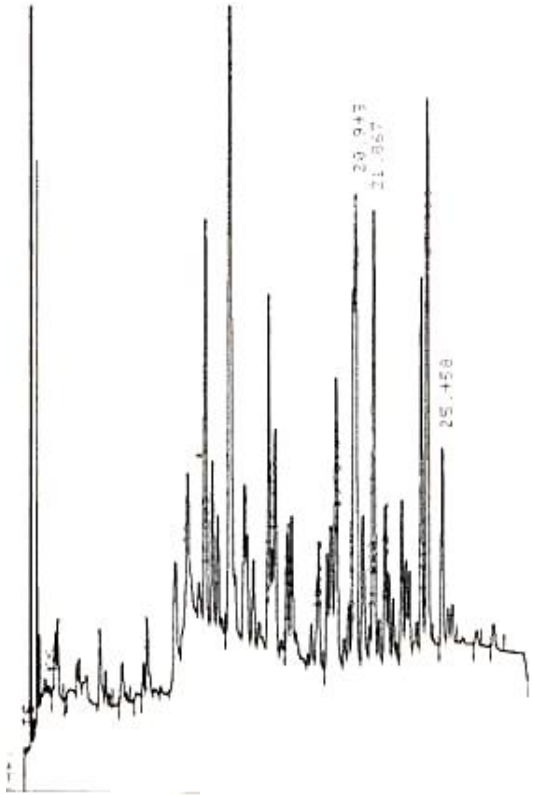
SPLIT

21 %



SPLITLESS

52 %



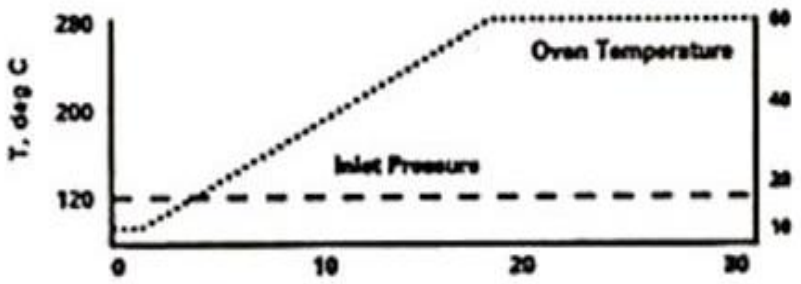
ON-COLUMN

100 %

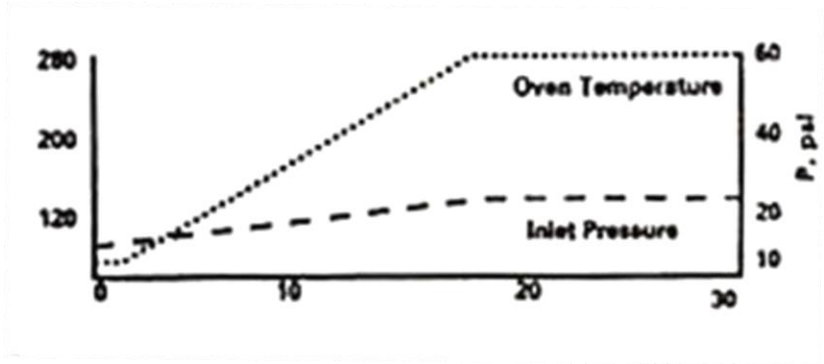
ELECTRONIC PRESSURE CONTROL (EPC) I

SPLIT, SPLITLESS, ON-COLUMN, detector gases

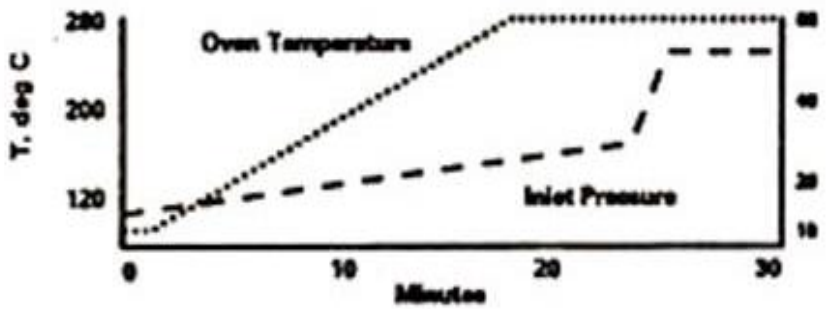
CONSTANT PRESSURE



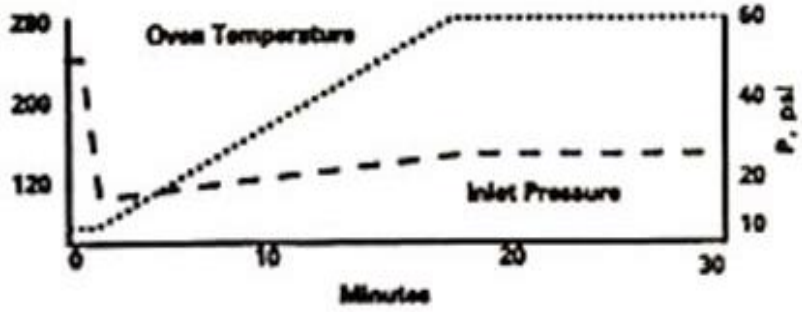
CONSTANT FLOW RATE



PRESSURE PROGRAMMING



PRESSURE PULSE FOLLOWED BY CONSTANT FLOW RATE



ELECTRONIC PRESSURE CONTROL (EPC) II

REASONS FOR EPC APPLICATION

- ***TEMPERATURE PROGRAMMING***
- ***LARGE VOLUME INJECTION***

↑ **TEMPERATURE** ⇒ ↓ RETENTION; ↑ DIFFUSIVITY
⇒ ↑ OPTIMUM OF LINEAR VELOCITY
(MAINTAINING OF CONSTANT EFFICIENCY)

↑ **TEMPERATURE** ⇒ ↓ LINEAR VELOCITY (L.V.)
⇒ **PRESURE PROGRAMMING ASSURES OPTIMUM OF L.V.**

EPC ADVANTAGES:

RT reproducibility improvement

Reduction of analysis time

Reduction of discrimination and decomposition of thermolabile compounds

Injection of larger volumes (upto 5 µl using pressure pulse)

Resolution improvement (narrower peaks)

ELECTRONIC PRESSURE CONTROL (EPC) III

Comparison of injection of different volumes

Volume: 1, 3, 5 µl (= 10, 30, 50 pg of each PCB in injection)
Column: DB-5 (60 m x 0.25 mm x 0.25 µm)

Relative response (%) = ($A_{n\ \mu l}$ / n * $A_{1\ \mu l}$) * 100

a) Constatnt pressure:

16 psi = 0.74 ml/min při 60 °C
= 0.33 ml/min při 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 při 60 °C
= 28.2 psi při 270 °C

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

ELECTRONIC PRESSURE CONTROL (EPC) IV

Comparison of injection of different volumes

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

150 kPa

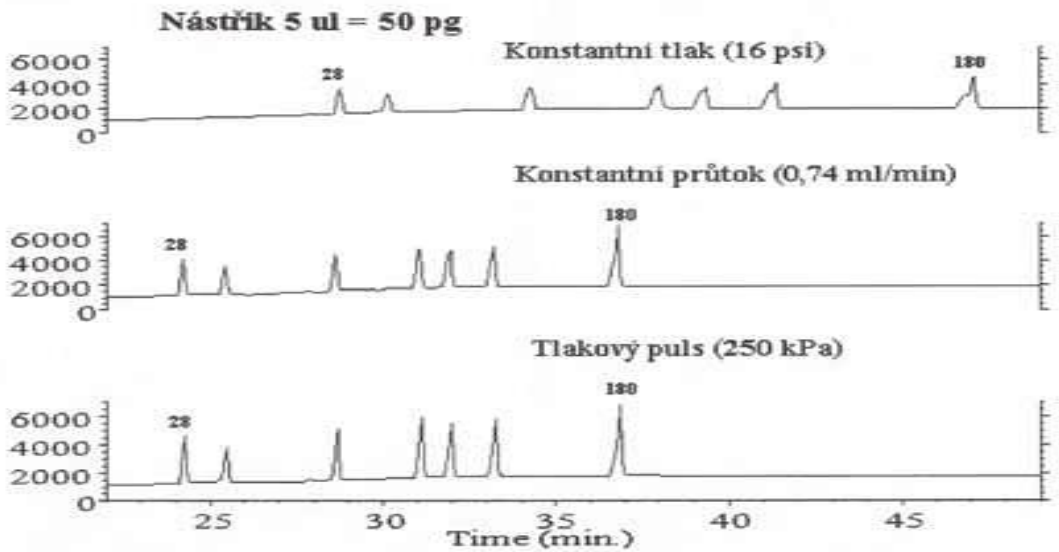
PCB	1 µL	3 µL	5 µL
28	100	89	91
180	100	85	94

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



ELECTRONIC PRESSURE CONTROL (EPC) V

REASONS FOR APPLICATION

Possibility to decrease LOD/LOQ

- e,g. 1 µl of solution 1000 µg/l = 50 µl of solution 20 µg/l

$\Rightarrow$ 50 x lower LOD/LOQ

Possibility to simplify sample preparation

- sample concentration step can be omitted (RVO, N_2)
- the sample weight / final extract volume ratio can be reduced (preparation of diluted extracts)

Possibility of online connection of methods

- sample extraction + GC or LC – e.g. SPE + GC/MS or LC/MS
- LC separation + GC separation

ELECTRONIC PRESSURE CONTROL (EPC) VI

Realizations

- the problem is the large amount of injected solvent, which causes bandbroadening of introduced zone and burdening of detectors

- various technical variants of the solution based on the removal of the solvent in front of analytical column are applied

Applied technical variants of injection

COOL ON-COLUMN - **COC**

COOL ON-COLUMN WITH SOLVENT VAPOR EXIT - **COC-SVE**

PROGRAMMED TEMPERATURE VAPORIZING INJECTOR - **PTV**

(COMBINED WITH SPLIT/SPLITLESS)

ELECTRONIC PRESSURE CONTROL (EPC) VII

TECHNIQUE	REALIZATION	VOLUME	LIMITATIONS
COC	precolumn ≈ 5 - 10 m	upto 0.1 ml	low-volatiles accumulation in column
COC-SVE	precolumn + SVE	upto 1 ml	low-volatiles accumulation in column
PTV	controlled injection speed packed or shaped liner cryo-cooling (CO_2)	upto 1 ml	loss of volatiles

ELECTRONIC PRESSURE CONTROL (EPC) VIII

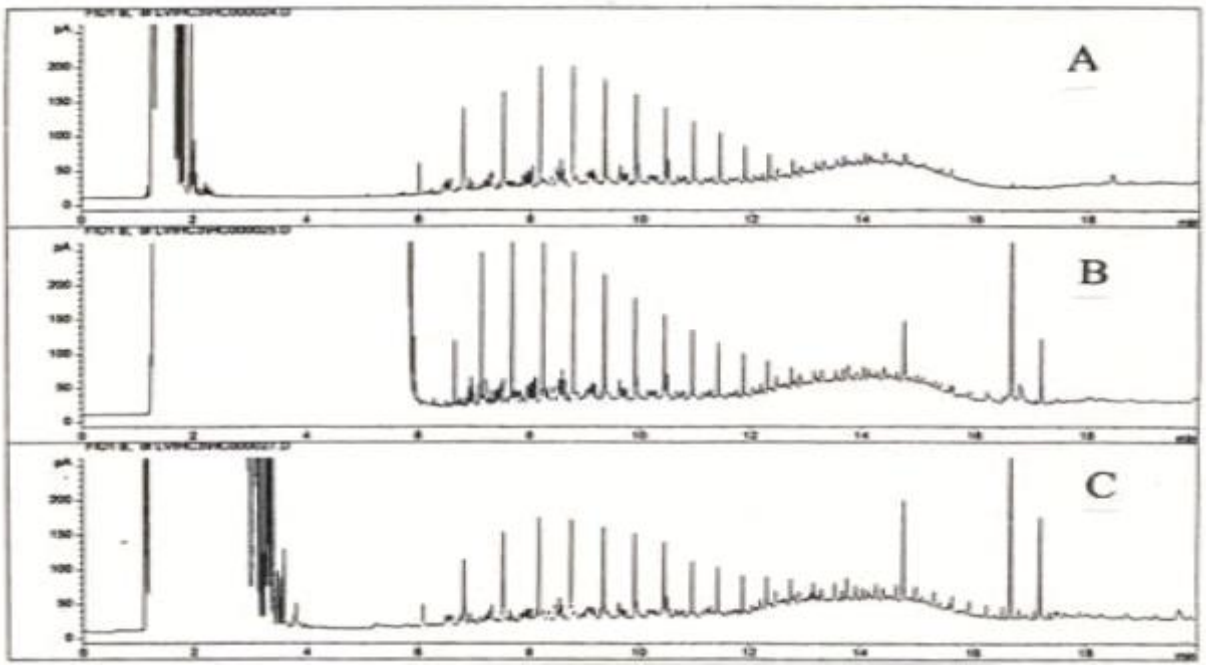
Comparison of COC techniques

Reference sample of mineral oil in hexane, GC/FID

A) COC – conc. 1 mg/ml, injection 1 µl

B) COC-precolumn - diluted 50x (=0,02 mg/ml), injection 50 µl

C) COC-SVE - diluted 50x (=0,02 mg/ml), injection 50 µl

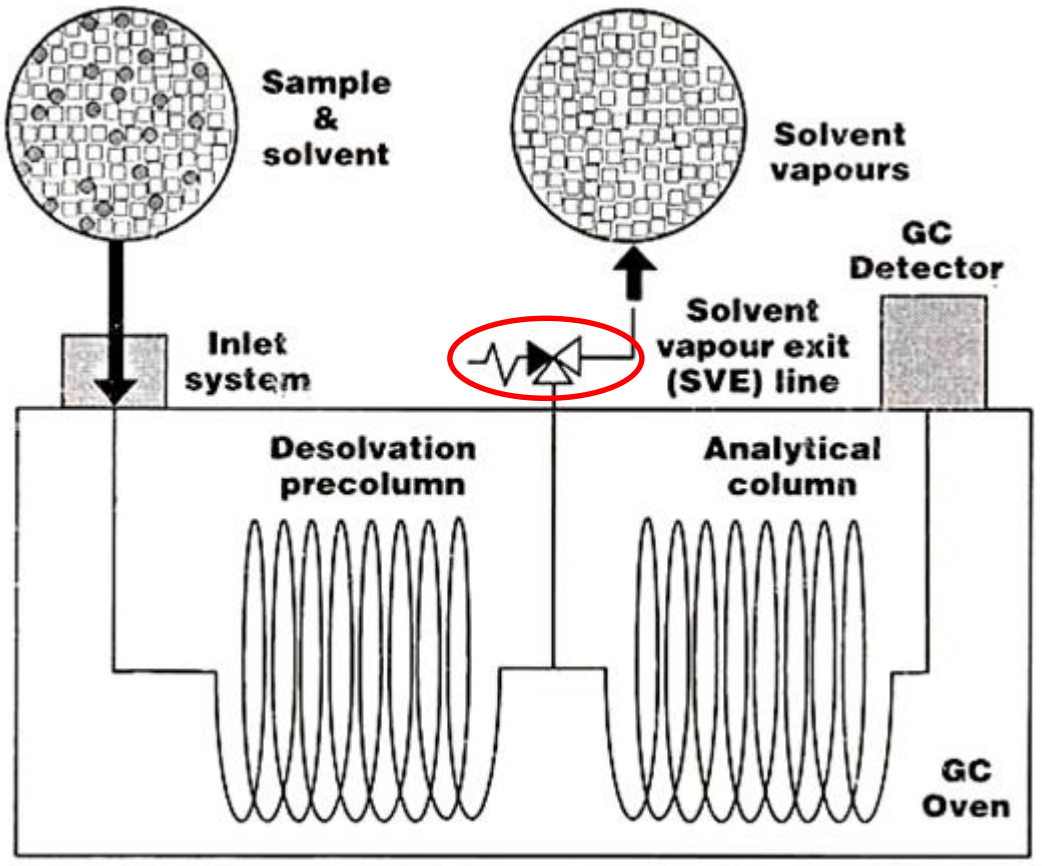


(F.David et al.: poster - 18. ISCC, 20.-24.5.1996, Riva del Garda, Italy)

COC-SVE REALIZATION

1. Solvent removal behind precolumn

2. Temperature programming after solvent removal (sensor, FID)

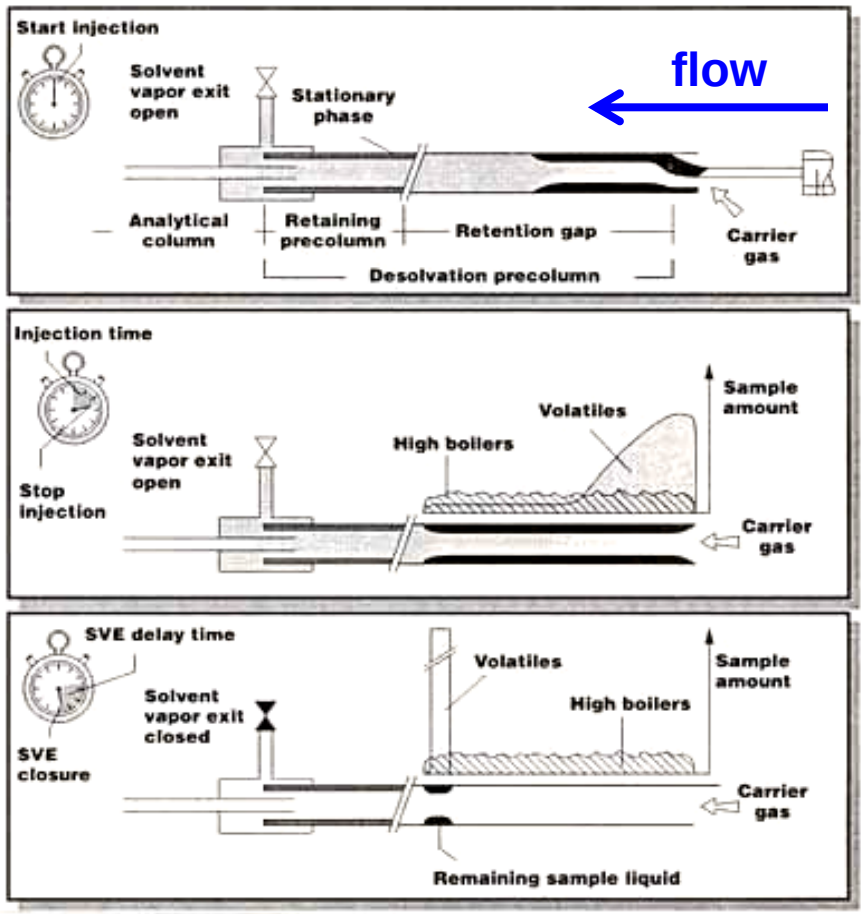


ELECTRONIC PRESSURE CONTROL (EPC) X

COC-SVE: DETAILED VIEW ON INJECTION

Minimal losses of volatiles

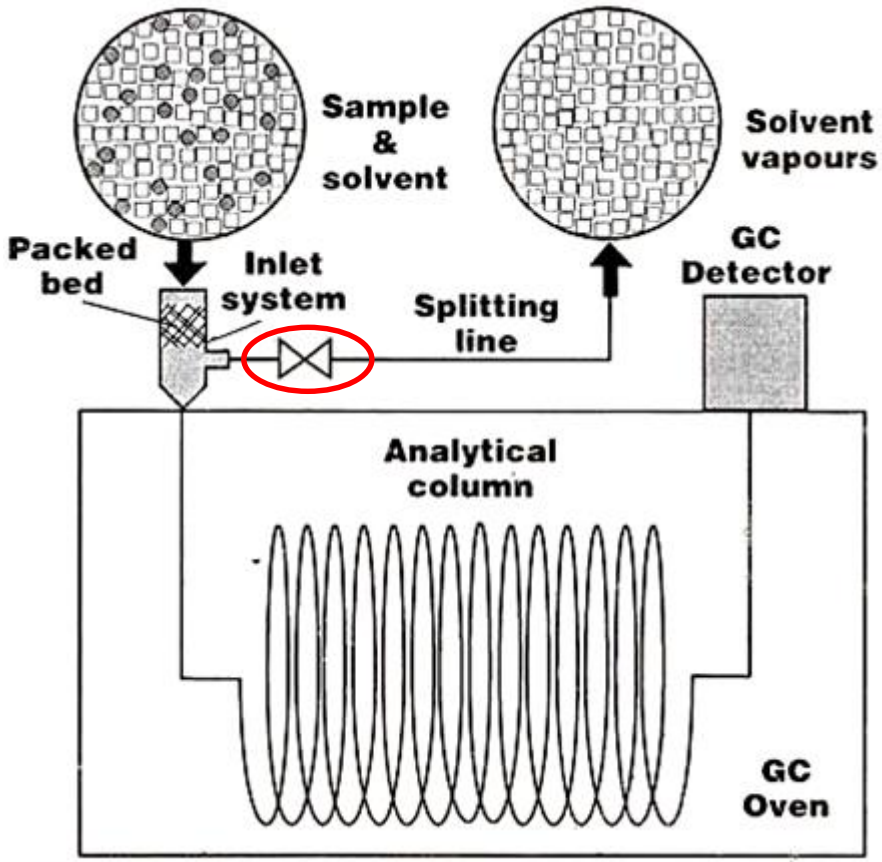
For „clean“ samples (contaminated precolumn causes peak tailing)



PTV REALIZATION

1. Solvent removal through split line

2. Temperature programming after solvent removal (sensor, FID)



PTV REALIZATION – TECHNICAL DEMANDS

Small internal volume of injector to facilitate rapid warm-up
Small internal volume of insert (same reason), approx. 15 -150 µl
Insert (liner) filled with glass wool or other material (shaped)
(retention of liquid sample - minimization of losses of highly volatile s.)

If injection speed = solvent evaporation speed,
it is theoretically possible to inject any amount of sample

Automated solution

1. Application of sensor on split line - enables optimization of solvent evaporation conditions (speed, temperature, flow rate), setting of the percentage of solvent removal
2. Adjustable injection speed

PTV INJECTION - SPLIT/SPLITLESS

1. Injection of liquid sample - into cold injector with open split
2. The solvent is carried into split line along with highly volatile substances
3. Split is closed and injector heats up rapidly - substances are transferred to column

Advantages and limitations

There is no discrimination in needle and injector and possible thermal degradation of compounds (compared to the classic SPLI/SPLITLESS)

Minimal problems with column contamination (need to change the insert filling regularly)

Losses of highly volatile analytes (compromise optimization is required: choice of insert filling, temperature and flow)

COC AND PTV – COMPARISON OF COLUMN CHANGES