

# Liquid Chromatography

BP 2017



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31 March 2017

BP 2017

Appendix III Chromatographic Separation Techniques

Appendix III D. Liquid Chromatography

## SYSTEM SUITABILITY

### Factors :

- composition, ionic strength, temp, pH of mobile phase
- flow rate, column dimensions, column temp, pressure
- stationary phase characteristics: type, particle size, porosity, specific surface area
- reverse phase and other surface-modification of stationary phase (end-capping, carbon loading etc.)

## SYSTEM SUITABILITY

- Symmetry factor : 0.8 – 1.5  
( in a related substances test or assay )
- % RSD : 6 replicate injections NMT 2.0  
( only to assays for formulated preparation )

- In **assay** of an **active substance** where the value is 100 percent for a pure substance, the maximum permitted relative standard deviation ( $Sr(\%)_{max}$ )

$$S_r (\%)_{max} = \frac{KB\sqrt{n}}{t_{90\%,n-1}}$$

$K$	=	constant (0.349), obtained from the expression $K = \frac{0.6}{\sqrt{2}} \times \frac{t_{90\%,5}}{\sqrt{6}}$ in which $\frac{0.6}{\sqrt{2}}$ represents the required percentage relative standard deviation after 6 injections for $B = 1.0$ ;
$B$	=	upper limit given in the definition of the individual monograph minus 100 per cent;
$n$	=	number of replicate injections of the reference solution ( $3 \leq n \leq 6$ );
$t_{90\%,n-1}$	=	Student's $t$ at the 90 per cent probability level (double sided) with $n-1$ degrees of freedom.

- Maximum permitted relative standard deviation does not exceed the appropriate value given in Table  
(does not apply to tests for related substances)

**Table 2.2.46.-1. – Repeatability requirements**

	Number of individual injections			
	3	4	5	6
<b><math>B</math> (per cent)</b>	<b>Maximum permitted relative standard deviation</b>			
2.0	0.41	0.59	0.73	0.85
2.5	0.52	0.74	0.92	1.06
3.0	0.62	0.89	1.10	1.27

## ADJUSTMENT OF CHROMATOGRAPHIC CONDITIONS

- Composition of the mobile phase

Minor components

$\pm 30\%$  relative or  $\pm 2\%$  absolute

( Whichever is the larger )

Not more than  $\pm 10\%$  absolute

### Exp.1

Specific ratio of 90:10

30%  $\rightarrow 10 \times 30/100 = 3\%$  (7 – 13)

2% absolute (8 – 12)

\*\* Larger relative adjustment range 7 – 13

Adjust within 93:7 to 87:13

Exp.2

Specific ratio of 95 : 5

30% →  $5 \times 30/100 = 1.5\%$  (3.5–6.5)

2% absolute (3 – 7)

\*\* Larger absolute adjustment range 3-7

Adjust within 97:3 to 93:7

Exp.3 Specific ratio of 60:35:5

- Second component 30% →  $35 \times 30/100 = 10.5\%$

permitted change 25 – 45

Mixture ranges 50 : 45 : 5 to 70 : 25 : 5

- Third component 30% →  $5 \times 30/100 = 1.5\%$

permitted change 3 – 7

Mixture ranges 58 : 35 : 7 to 62 : 35 : 3

## ADJUSTMENT OF CHROMATOGRAPHIC CONDITIONS

- pH of the aqueous component of the mobile phase
  - :  $\pm 0.2$  pH
  - or  $\pm 0.1$  pH when non-ionisable substances

## ADJUSTMENT OF CHROMATOGRAPHIC CONDITIONS

- Concentration of salts in Buffer
  - :  $\pm 10\%$

Exp. 1.0 M

permitted change 0.9 to 1.1 M

## ADJUSTMENT OF CHROMATOGRAPHIC CONDITIONS

### Column parameters

#### Stationary phase

- No change of the identity of the substituent of the stationary phase  
(eg. No replacement of C18 by C8)
- Particle size : decrease 50%

No increase \*\*

## ADJUSTMENT OF CHROMATOGRAPHIC CONDITIONS

### Column dimension

- Length :  $\pm 70\%$
- Internal diameter :  $\pm 25\%$

## ADJUSTMENT OF CHROMATOGRAPHIC CONDITIONS

- Flow rate :  $\pm 50\%$

\*\* A larger adjustment when changing the column dimensions  
(see formula)

When column dimension changed

$$F_2 = F_1 \frac{l_2 d_2^2}{l_1 d_1^2}$$

$F_1$  = flow rate indicated in the monograph

$F_2$  = adjusted flow rate

$l_1$  = length of the column indicated in the monograph

$l_2$  = length of the column used

$d_1$  = internal diameter of the column indicated in the monograph

$d_2$  = internal diameter of the column used



## ADJUSTMENT OF CHROMATOGRAPHIC CONDITIONS

- Temperature :  $\pm 10^{\circ}\text{C}$
- Detector wave length : No adjustment \*\*
- Injection volume : May be decrease  
No increase \*\*

## ADJUSTMENT OF CHROMATOGRAPHIC CONDITIONS

### Gradient elution

#### Column parameter

- No change of the identity of the substituent of the stationary phase
- Particle size : no adjustment \*\*
- Length :  $\pm 70\%$
- Internal diameter :  $\pm 25\%$

## ADJUSTMENT OF CHROMATOGRAPHIC CONDITIONS

### Gradient elution

#### Mobile phase

- pH of the aqueous component of the mobile phase  
: No adjustment \*\*
- Concentration of salts in the buffer component  
: No adjustment \*\*

## ADJUSTMENT OF CHROMATOGRAPHIC CONDITIONS

### Gradient elution

- Flow rate  
may be adjusted following equation

$$F_2 = F_1 \frac{l_2 d_2^2}{l_1 d_1^2}$$

## ADJUSTMENT OF CHROMATOGRAPHIC CONDITIONS

### Gradient elution

- Temperature :  $\pm 5^{\circ}\text{C}$
- Detector wavelength : No adjustment \*\*
- Injection volume : may be decrease

No increase \*\*

Thank you

