



Fast Analysis of DA and 5-HT in Small Sample Volumes

ALEXYS® 100 LC-EC system

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Introduction

The fast analysis of DA and 5-HT in small sample volumes was investigated. Reproducibility, linearity and a special injection method were studied.

Fast LC analysis

Under fast LC conditions, the total analysis time of DA and 5-HT analysis could be decreased to less than 2 minutes.

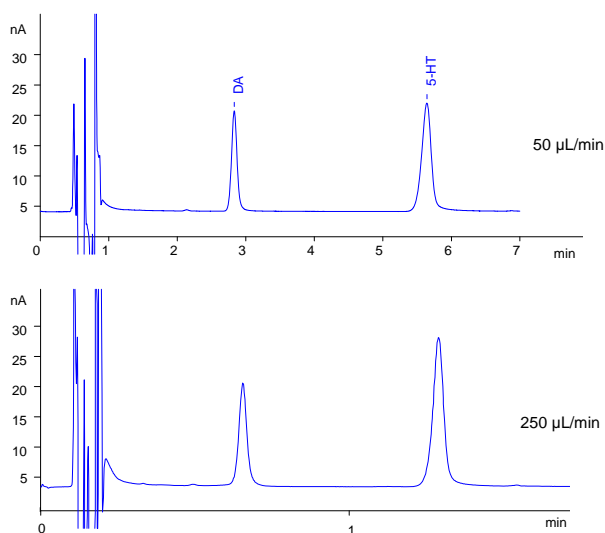


Fig. 1. Analysis of 5 µL 100 nM DA and 5-HT in 10 mM perchloric acid, analyzed using different flow rates.

Small samples, optimised injection

When only 5 µL sample is available for analysis, the injection method has to be optimal for best sensitivity. This is achieved by combining a small sample needle, a small sample loop and a customised injection program. In principle, the program consists of the following steps:

- aspiration of 5 µL sample

- transport of the sample plug to the sample loop using transport fluid

When using the optimal amount of 5 µL transport fluid, the actual on-column sample load is equivalent to a 3 µL injection. The loss of response is due to dilution of the sample during transport through the tubing.

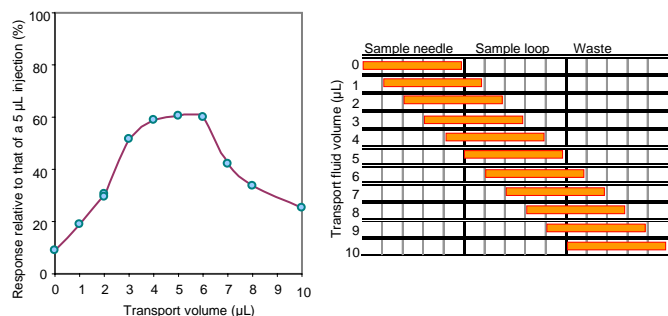


Fig. 2. Left-side: Relative response of customised injection using 5 µL sample only and different volumes of transport fluid (data based on peak area and peak height of DA and 5-HT). Right-side: schematic display of sample allocation in autosampler tubing using different volumes of transport fluid.

Method

HPLC ALEXYS 100 'Microdialysis I' (part no 180.0060)
 Flow cell Sencell™ with 5 mm GC electrode and salt bridge reference electrode
 V_{injection} 5 µL (user program)
 T_{oven} 35 °C for separation and detection
 Flow rate 250 µL/min

Reproducibility and linearity

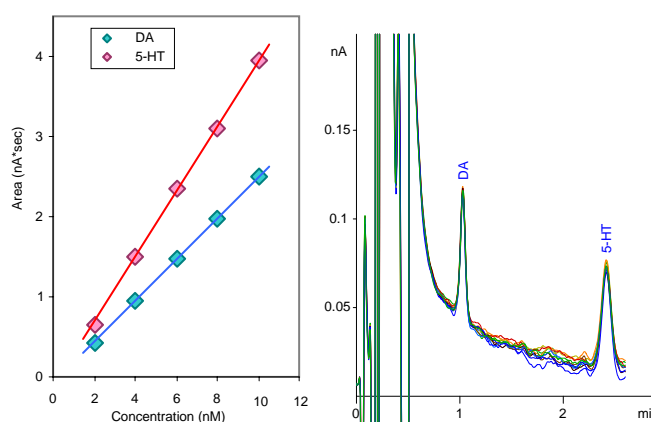


Fig. 3. Linearity plot (l) and overlay (r) of 10 chromatograms of 1 nM DA and 5-HT in acidified Ringer.

Table 3. Reproducibility parameters (n=10) from 1 nM (see Fig. 3) and 100 nM standard.

	Conc nM	Tr (min)		Area (nA*sec)		Height (nA)	
		avg	%RSD	avg	%RSD	avg	%RSD
DA	1	1.01	0.16	24.7	1.2	7.3	1.0
	100	1.03	0.13	0.25	3.6	0.072	1.6
5-HT	1	2.40	0.15	40.5	0.9	6.0	1.0
	100	2.42	0.08	0.38	4.5	0.057	3.1

The RSD for 10 replicate injections of 100 nM standard was 0.2% or better for retention time and 1.2 % or better for peak area. For the concentration of 1 nM, which is closer to the detection limit, the RSD was better than 5% for peak area. A good linear response (correlation coefficient >0.999) was found in the concentration range of 2-10 nM. Standards in acidified Ringer.

Fast analysis near LOD

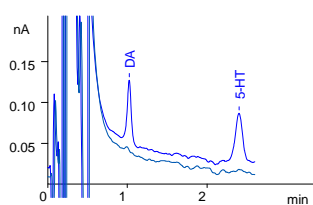


Fig. 4. Analysis of blank and 1 nM DA and 5-HT in acidified Ringer

Conclusion

The data demonstrates that the use of 5 µL sample and a higher flow rate results in reproducible and linear analysis of DA and 5-HT in less than 3 minutes with a detection limit of about 0.1 nM DA and 5-HT.