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Fused-core HPLC method development implemented in a short-term stability study of Triple IT solution

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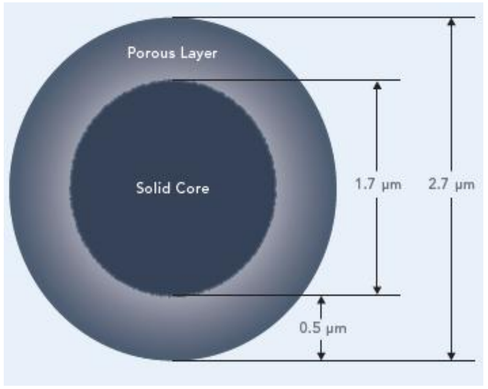
INTRODUCTION



For the majority of children with acute lymphoblastic leukemia (ALL), prophylactic treatment of the central nervous system consists in part of a triple intrathecal (Triple IT) therapy, *i.e.* a combination of cytarabine (CB), methotrexate (MTX) and methylprednisolone sodium succinate (MPSS). This combination product is prepared *ex-tempore*. However, no in-use shelf-life under defined storage conditions has yet been established. During these stability studies, a large number of samples are generated, thus creating the need for a fast, accurate and selective analytical method. Newly developed fused-core HPLC stationary phases (HALO® columns) are suited for these high-throughput purposes. Due to their small particle size and unique particle technology, with 0.5 µm porous shell fused to a solid core particle, these columns allow fast and high performance separations. Subsequently, this new column technology was chosen for the development of a stability-indicating HPLC method to be used during a short-term stability study of the Triple IT solution [1].

Objective ➔ (i) development of stability-indicating HPLC-method and (ii) method evaluation.

EXPERIMENTAL

HPLC parameters:

- Column: HALO C18 (4.6×150 mm, 2.7 µm) + guard
- Mobile phase A: 0.1% glacial acetic acid in H_2O
- Mobile phase B: 0.1% glacial acetic acid in ACN
- Column / sample compartment temp.: 30 C / 15 C
- Injection volume: 10 µl
- Detection: PDA 190-400 nm, detection @ 240 nm

Gradient program:

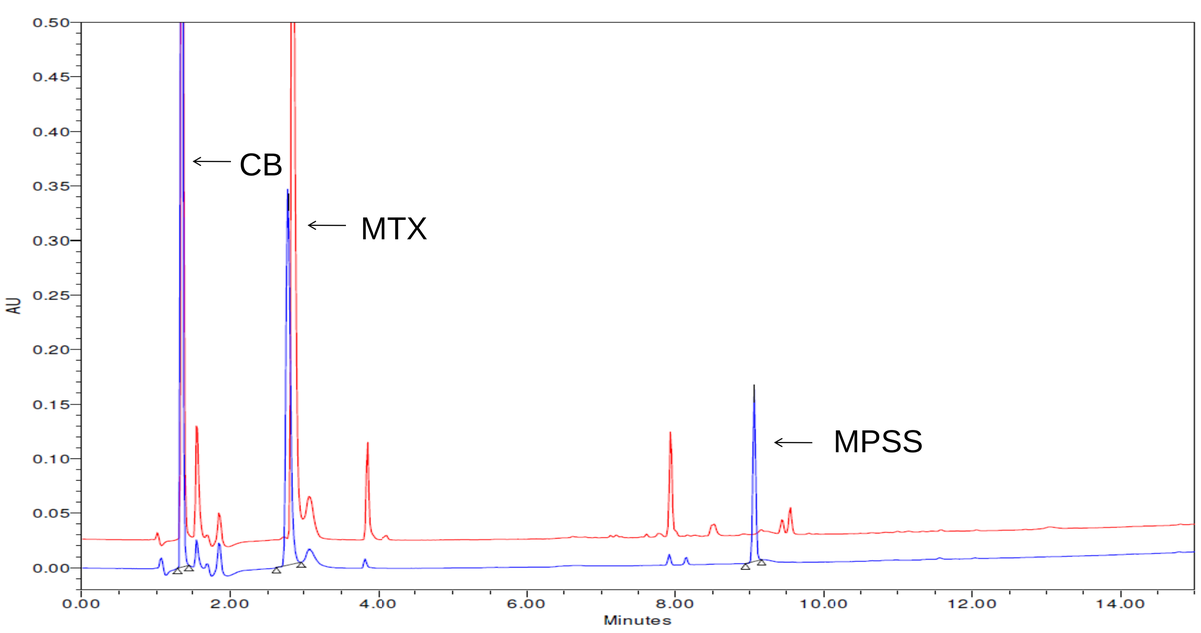
#	Time (min.)	Flow rate (ml/min)	%A	%B	Remarks
1	Different time intervals (5 to 30 min.)	1	90	10	Analysis
2			10	90	
3	3 min.		90	10	Column rinsing + equilibration
4	12 min.		90	10	

Solutions:

- Individual components unstressed
- Individual components stressed: (40 C and 80 C, up to 29 hrs.)
- Mixture of components unstressed
- Mixture of components stressed: (40 C and 80 C, up to 29 hrs.)

RESULTS and DISCUSSION

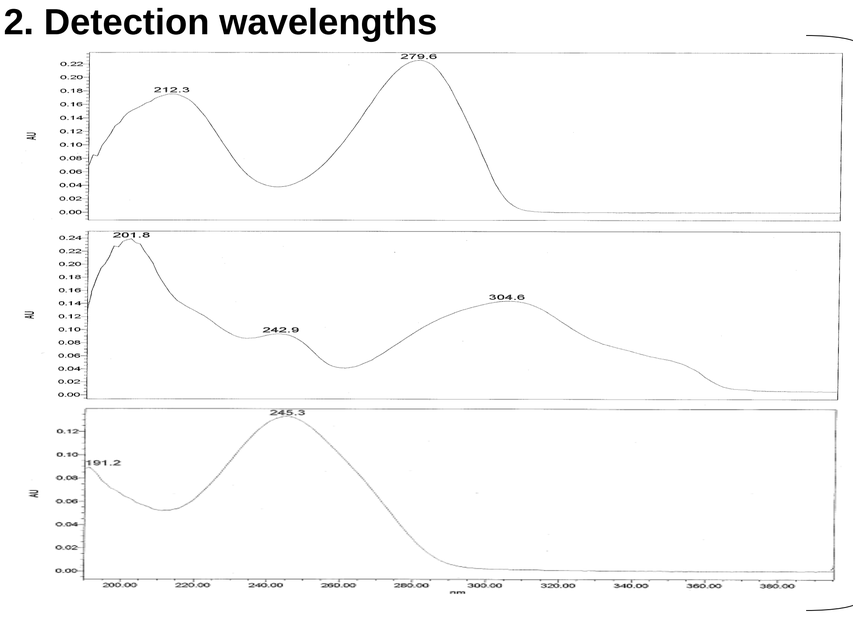
1. Gradient time interval: 15 min.



Mixture of components unstressed: 5 C, 29 hrs.
Mixture of components stressed: 80 C, 29 hrs.

- **Single** HPLC method capable of separating the three structurally different Triple IT components.
- **Fast** separation (total run time = 30 min): Reduction of solvent, time and hardware consumption
- Sufficient **resolution** between individual components and related degradation products.
- Peak purity analyses suggests **pure** peaks: (Triple IT components and related degradants)

2. Detection wavelengths



CB: 280 nm

MTX: 280 nm

MPSS: 240 nm

3. Method evaluation

Parameter	CB	MTX	MPSS
Linearity (R^2; 80-100-120% l.c.)	1.0000	0.9992	1.0000
Repeatability (%RSD; 100% l.c.; n = 3)	0.464	1.352	0.155
LoQ (% l.c.)	0.03	0.07	0.05

CONCLUSIONS

The development of a stability-indicating high-throughput HPLC method for three Triple IT components (CB, MTX and MPSS) was done using a fused-core (HALO®) stationary phase.

- 15 min. gradient ➔ separation of individual components and related degradation products.
- Method verification ➔HPLC method fit for use in a short-term Triple IT storage stability protocol.

REFERENCES

[1] M. D'Hondt, E. Vangheluwe, S. Van Dorpe, et al. Stability of *ex-tempore* prepared Triple intrathecal solution consisting of cytarabine, methotrexate and methylprednisolone sodium succinate. American Journal of Health-System Pharmacy, submitted for publication.