



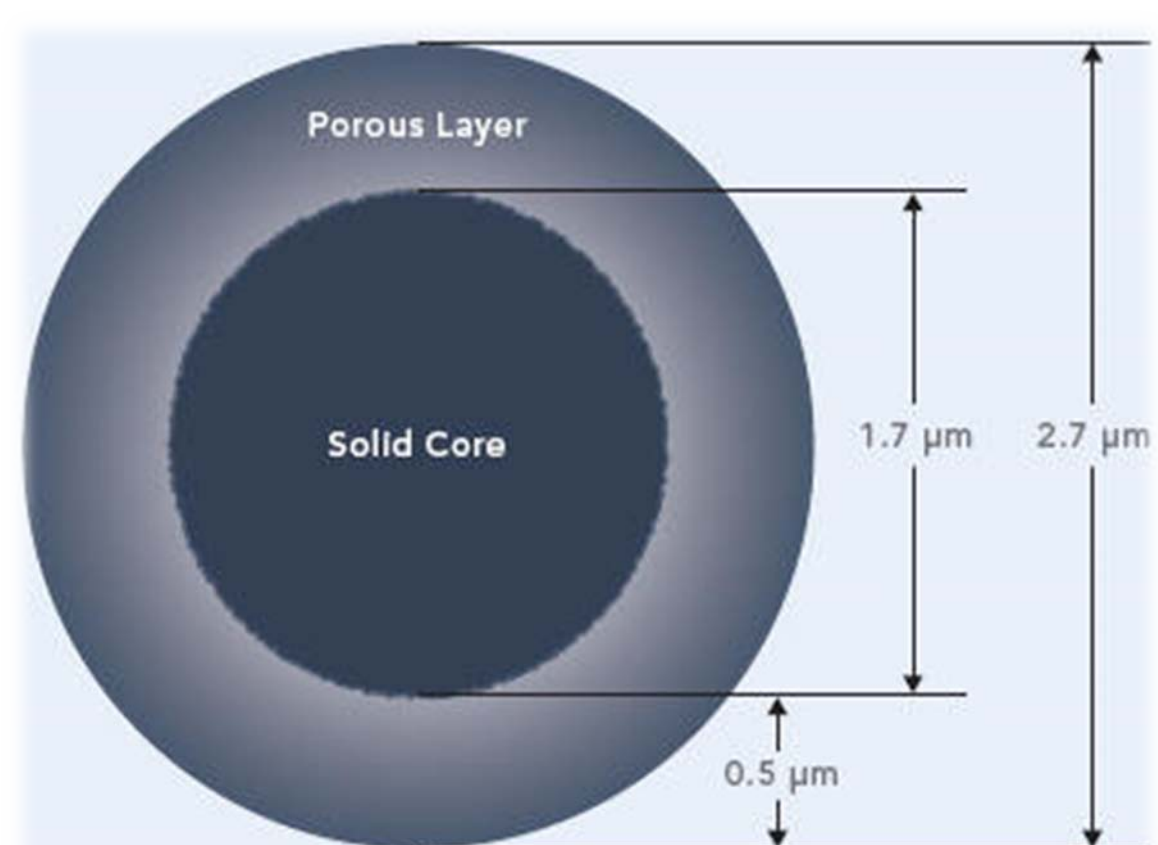
Influence of injection volume and solvent strength on spilanthol chromatography using RP fused-core amide stationary phase

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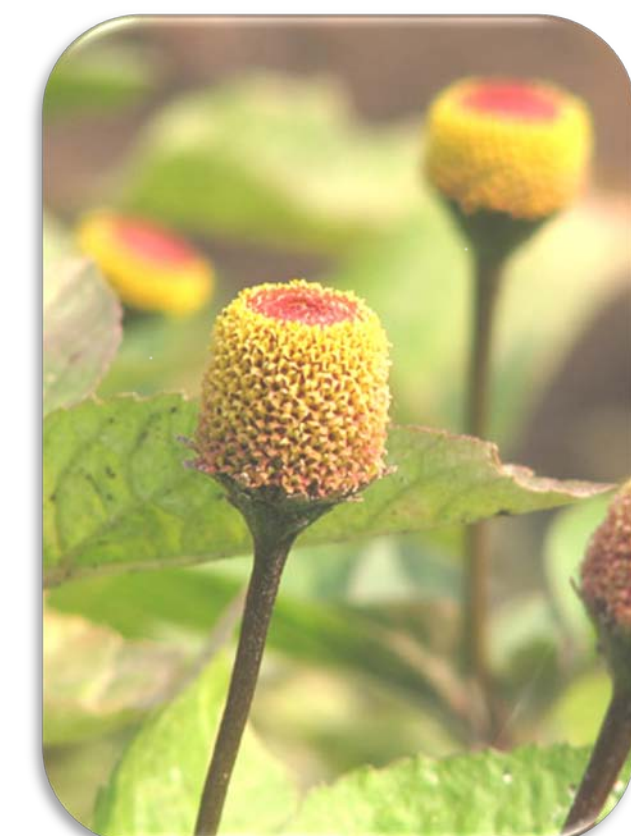
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INTRODUCTION



For high-throughput purposes, newly developed fused-core HPLC stationary phases (HALO[®] columns) have attracted the interest of the chromatographic community. Due to their small particle size and unique particle technology with 0.5 μm porous shell fused to a solid core particle, these columns create fast and high performance separations. Spilanthol, present in *i.a.* *Spilanthes acmella*, is a typical model *N*-alkylamide possessing bio-activity [1], can be analyzed in reversed-phase mode. To get insights in the optimal HPLC conditions including sufficiently low detection limits, the influence of injection volume and sample solvent on chromatographic HPLC-UV characteristics using a HALO[®] RP-amide column was investigated.



EXPERIMENTAL

Settings

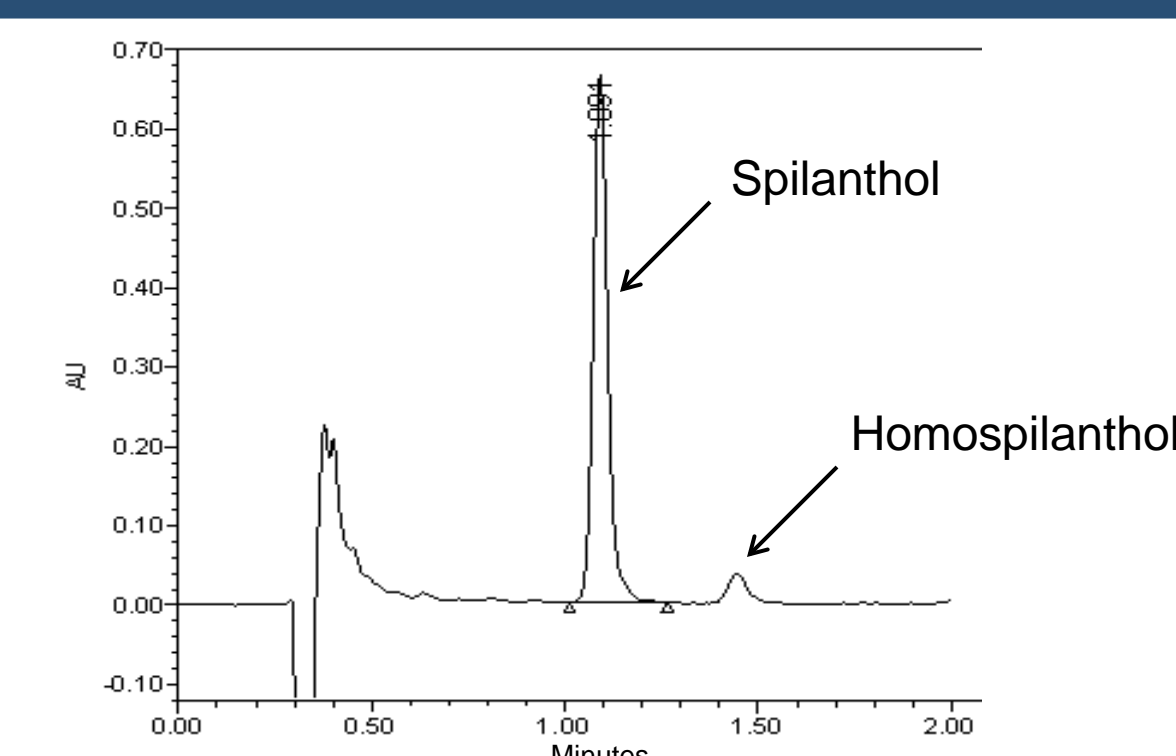
- **Column:** HALO[®] RP-amide column (4.6 × 50mm, 2.7 μm)
- **Mobile phase:** 1% F.A. in MeOH/H₂O (70/30, V/V)
- **Flow:** 1.5 ml/min
- **Run time:** 2 minutes
- **UV detection:** 237 nm

Variables

- **Injection volume:** 2 to 100 μL
- **Sample solvent:** PBS and MeOH/H₂O (70/30, V/V)

Responses

Chromatographic characteristics: retention time, area, height, theoretical plates, symmetry factor and limit of detection



RESULTS and DISCUSSION

Figure 1 shows the responses for each of the applied injection volumes, expressed relative to 2 μL (the smallest injection volume, which was taken as 100% reference). Figure 2 displays the LoD, expressed in concentration (μg/ml) and as mass injected on the column (ng).

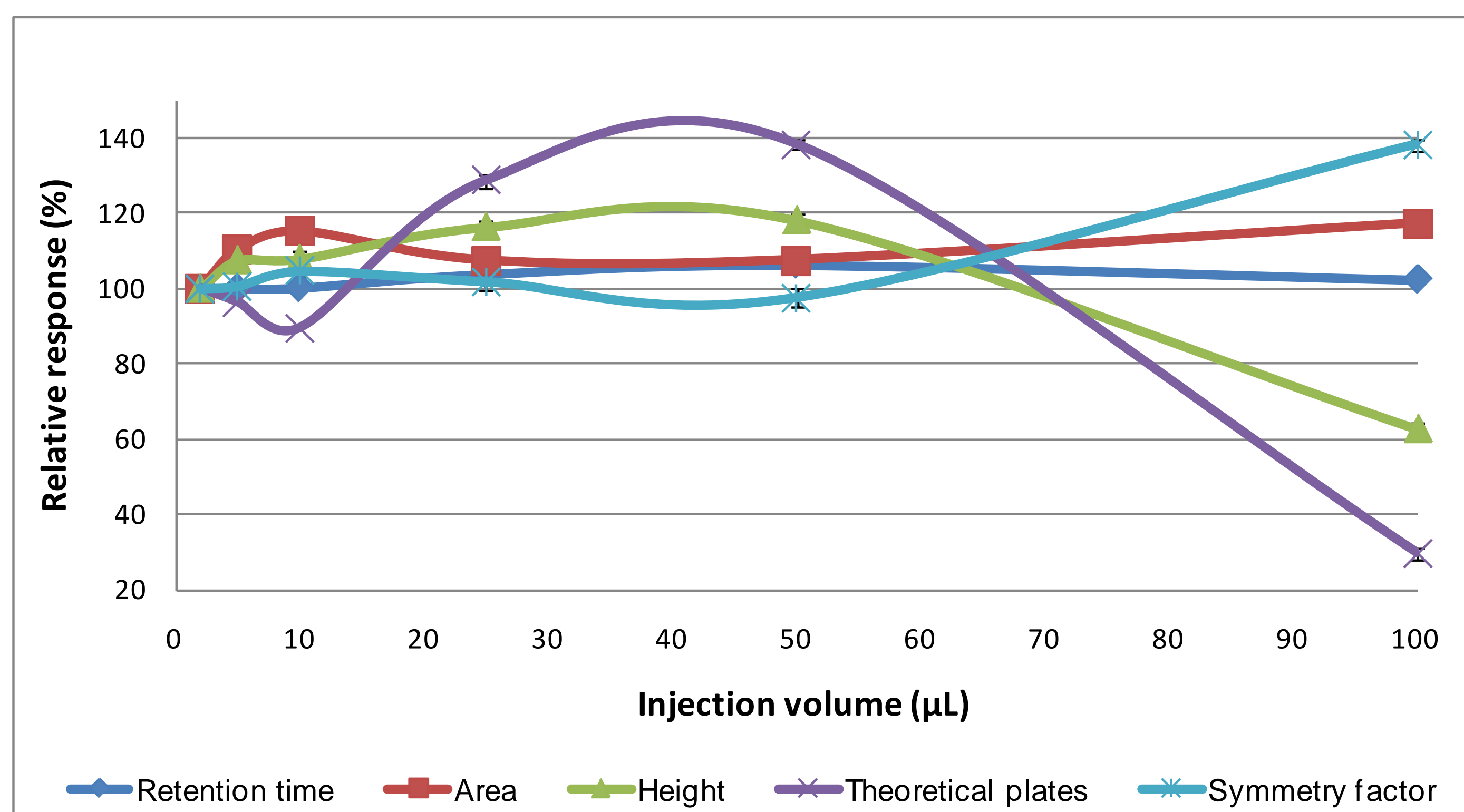
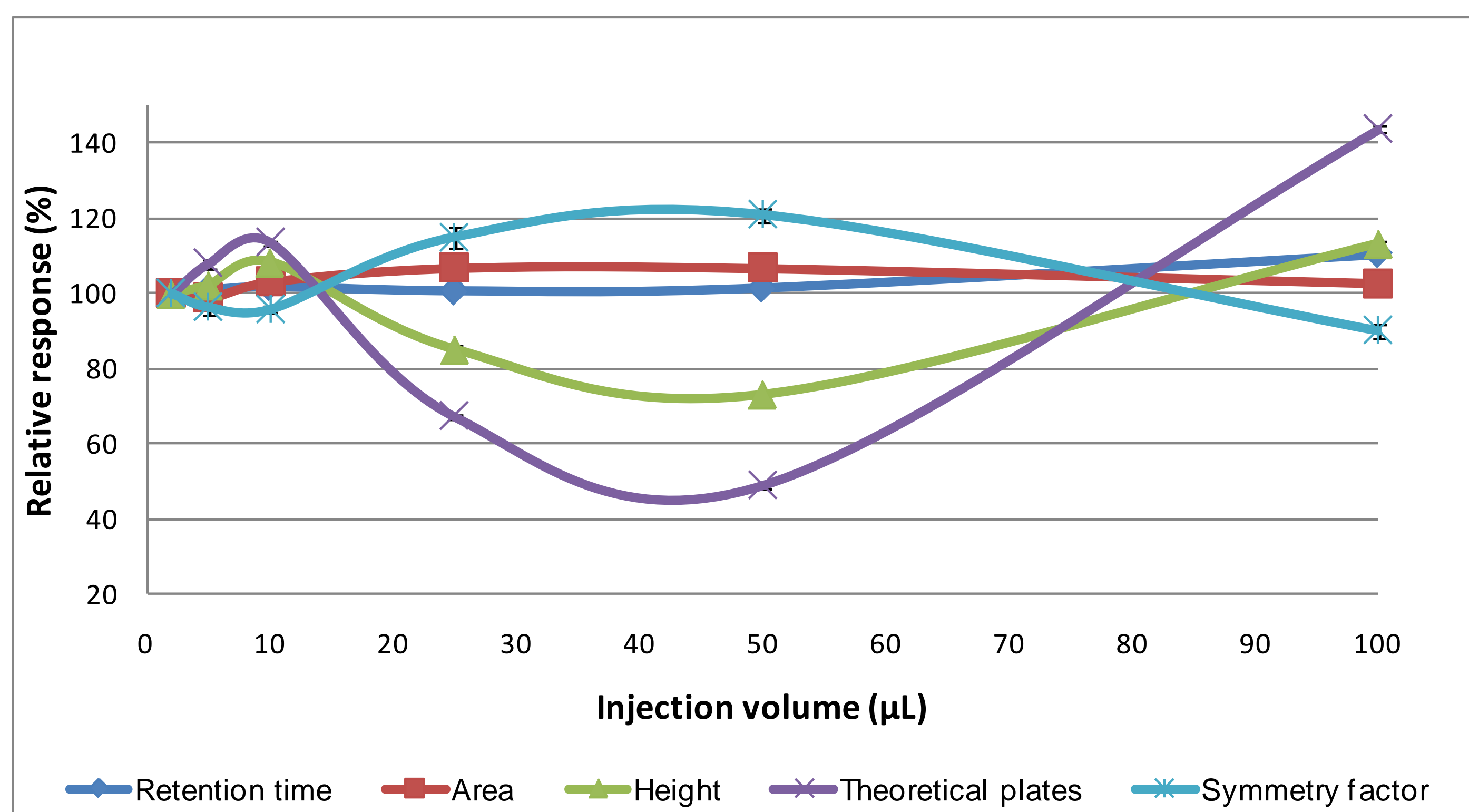


Figure 1: Chromatographic characteristics of PBS (left) and methanol-based (right) sample relative to injection volume of 2 μL

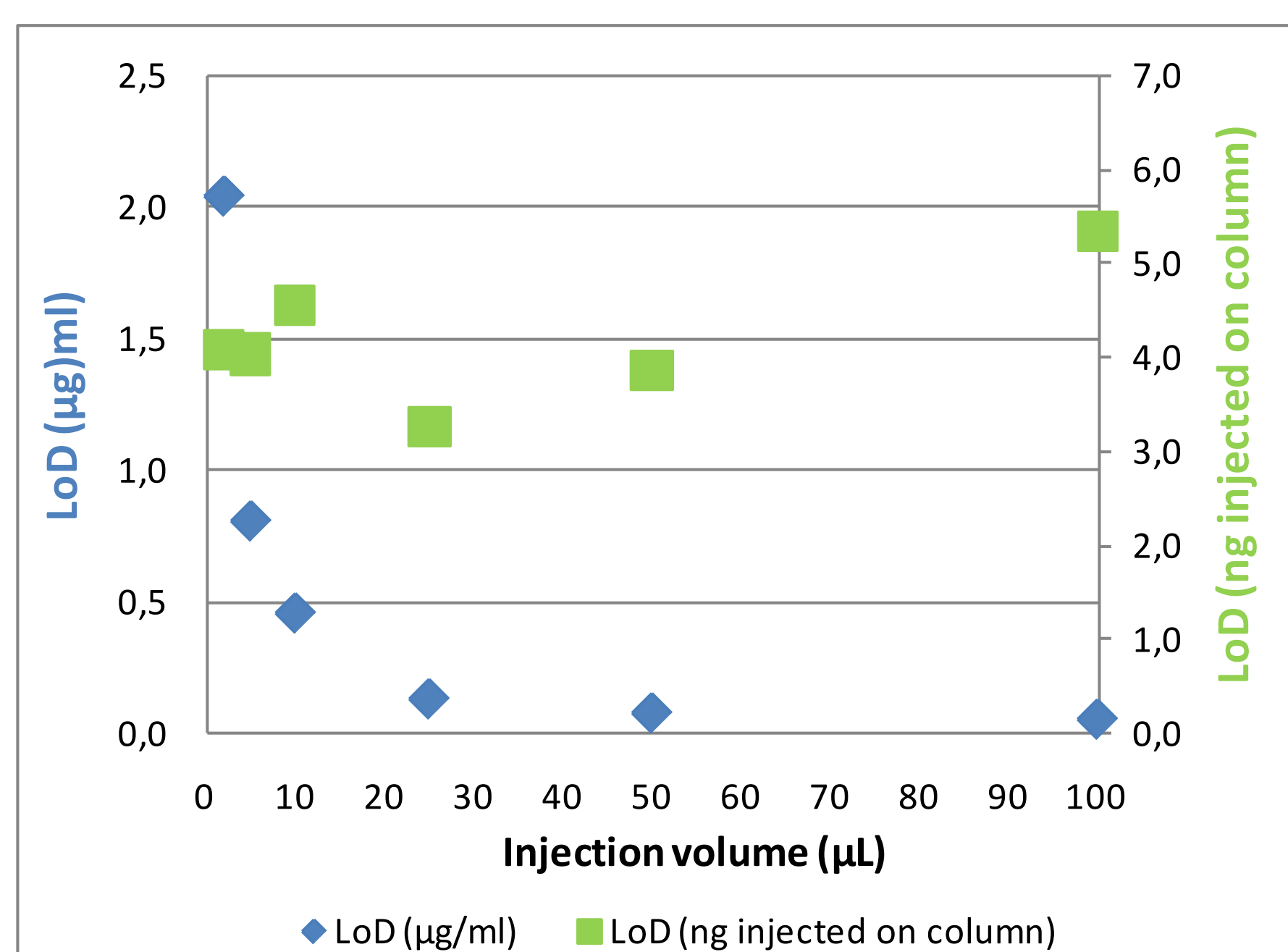
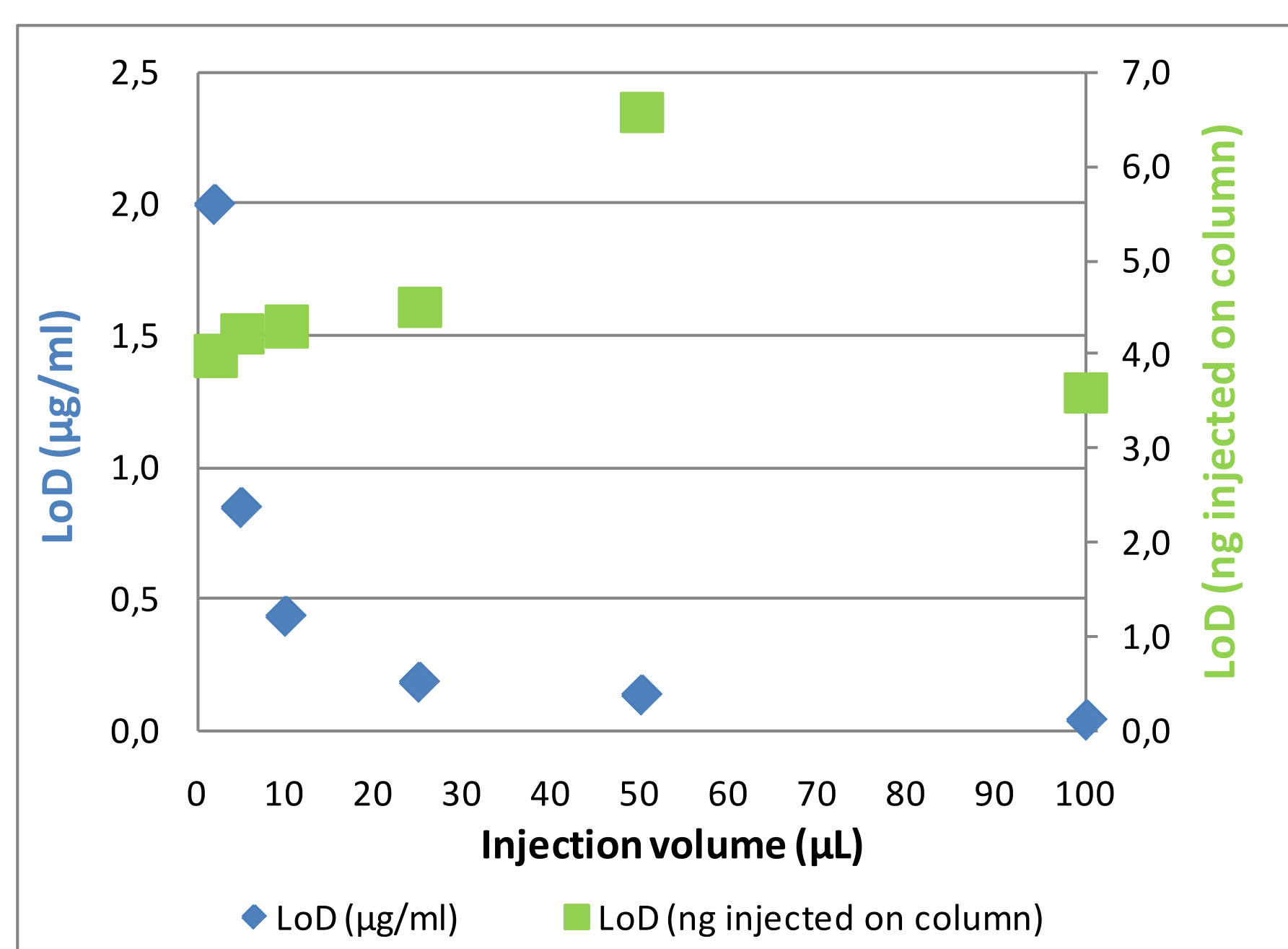


Figure 2: LoD for different injection volumes of PBS (left) and methanol-based (right) sample

- An injection volume of 100 μL, using PBS-based samples will be applied for FDC analyses.
- The injection volume and sample solvent are critical method parameters which have to be defined in method description/development/validation within the QbD frame.
- Further mathematical modelling is on-going.

CONCLUSIONS

Both the injection volume and the relative strength of the sample solvent have a significant contribution to the chromatographic characteristics using fused-core stationary phases [2]. In particular, a 100 μL injection volume with PBS-based sample solvent is the optimal condition for sensitive and efficient chromatography.

REFERENCES

- [1] J. Boonen, B. Baert, N. Roche, C. Burvenich and B. De Spiegeleer. Transdermal behaviour of the *N*-alkylamide spilanthol (affinin) from *Spilanthes acmella* (Compositae) extracts, 2010, 127 (1), 77-84.
- [2] J. Boonen and B. De Spiegeleer. The injection volume: a critical quality attribute for fused-core stationary phases. Publication in preparation.