

INTRODUCTION

The plate purification service at AstraZeneca R&D Mölndal is now implementing SFC-MS as an alternative to RP HPLC-MS. The use of SFC will require an additional column screen, and this poster focuses on selecting a column set as diverse as possible for the screen.

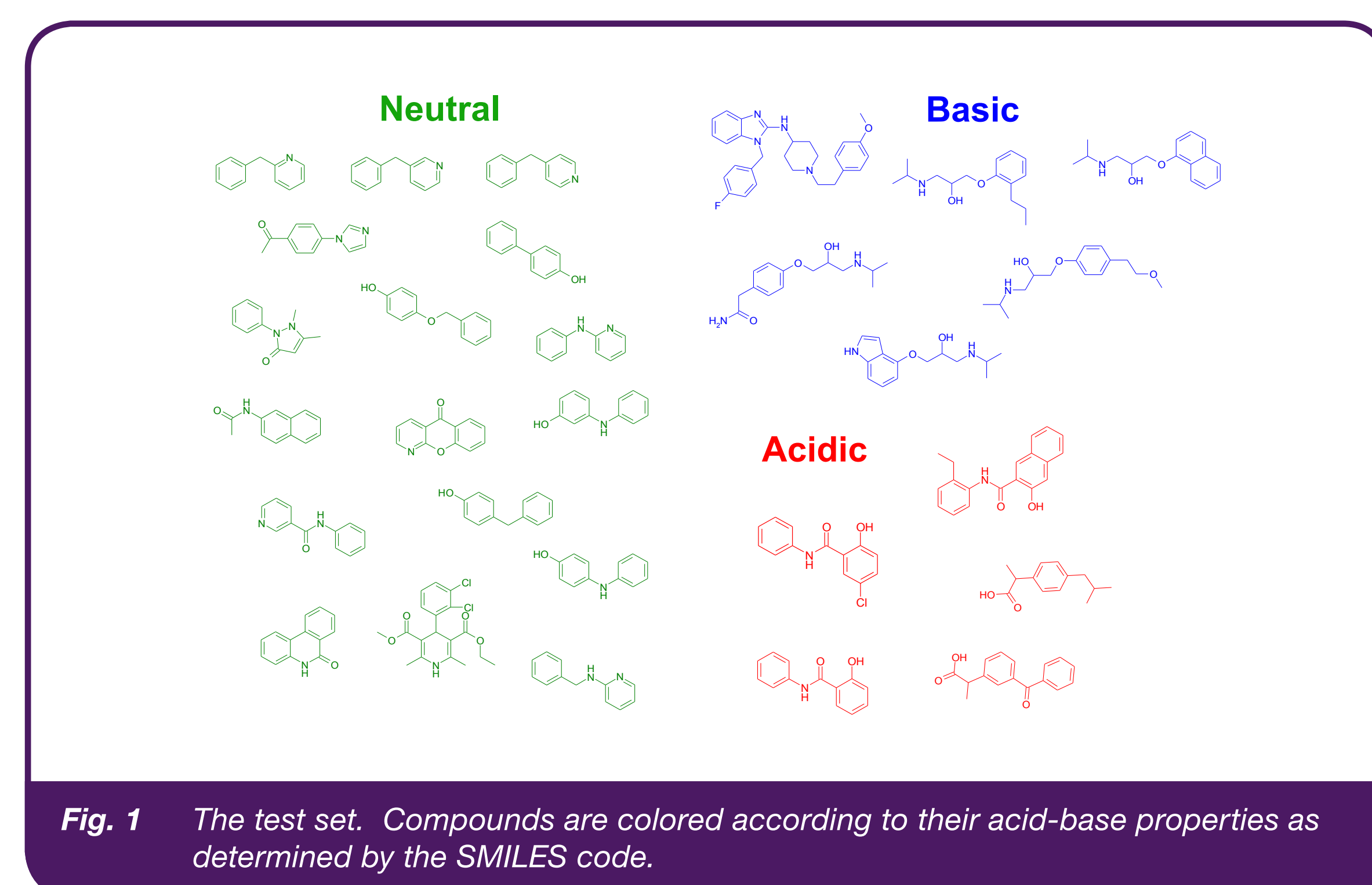
The analytical experiments have been performed using a Waters Resolution X5 SFC MS System, which allows parallel screening of five columns.

EXPERIMENTAL

The analyses were performed on a Waters Resolution X5 SFC MS System at 40°C and 120 bar using a 10 min gradient from 10-45% MeOH:DEA 100:0.5. The flow was 4 ml/min in single mode and 20 ml/min in parallel mode. Ions were identified using positive ESI and with a make-up flow of 10 mM formic acid in MeOH:H₂O 90:10. Additional information can be given by the author.

SELECTING A COLUMN SET FOR SCREEN

A test set was prepared using drug-like compounds with varying properties such as size, lipophilicity and acid-base properties (Fig. 1).



The compounds in the test set were analyzed in parallel mode using the same method on 19 different stationary SFC phases (Table 1).

Waters Viridis 2-EP

Waters Viridis SIL

Phenomenex Luna HILIC

Phenomenex Synergi Polar-RP

Princeton DNP

Kromasil NH2

Kromasil SIL

Kromasil Diol

Kromasil Phenyl

Kromasil CN

Princeton 4-EP

Princeton PPU

Princeton Pyridine Amide

Princeton Benzamide

Princeton PA

Princeton Benzene Sulfonamide

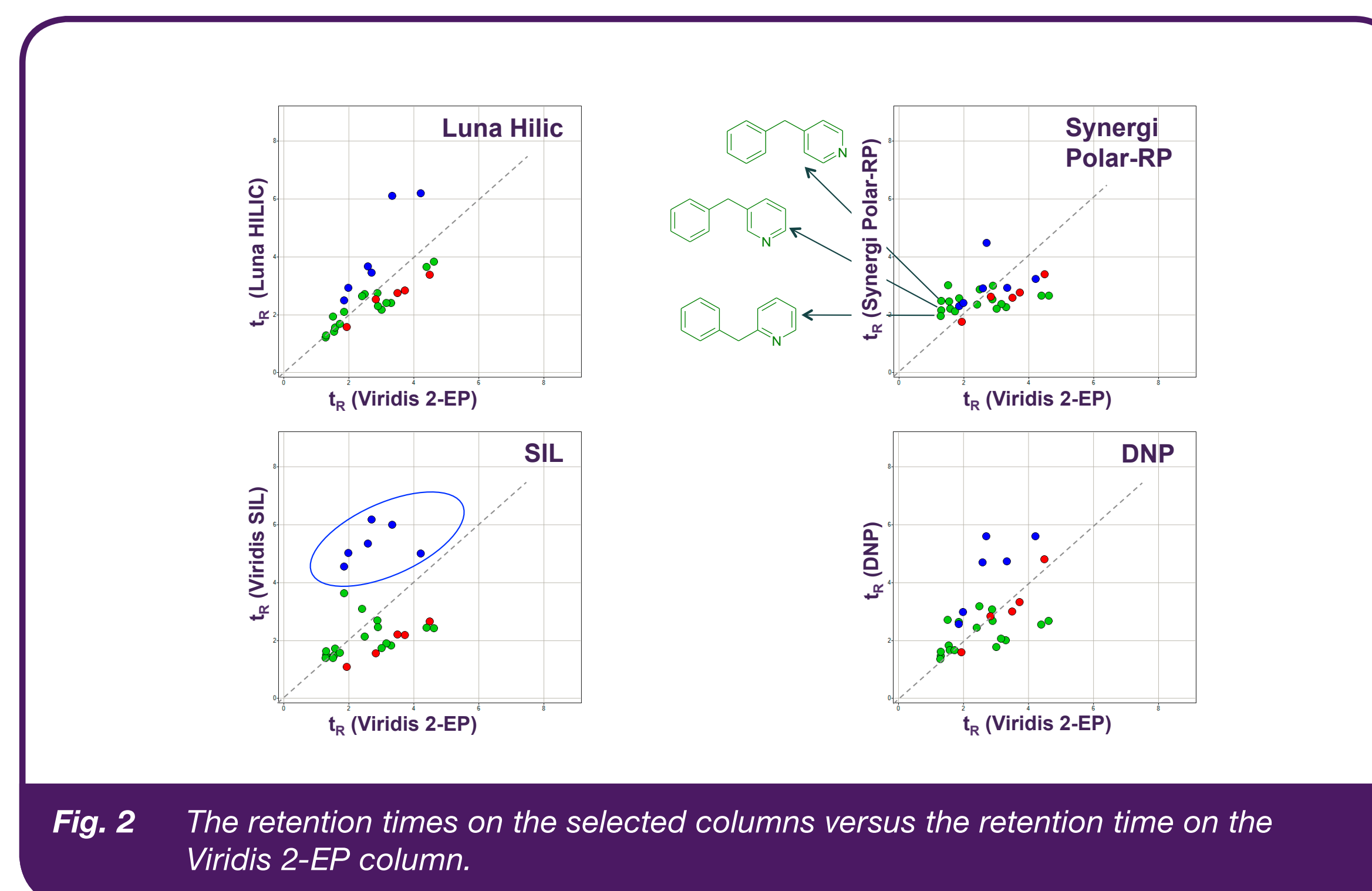
Princeton 2CN-DIOL

Princeton DEAP

Princeton PFP

Table 1 Stationary phases (column dimension: 250 x 4.6 ID mm). Selected columns are in bold.

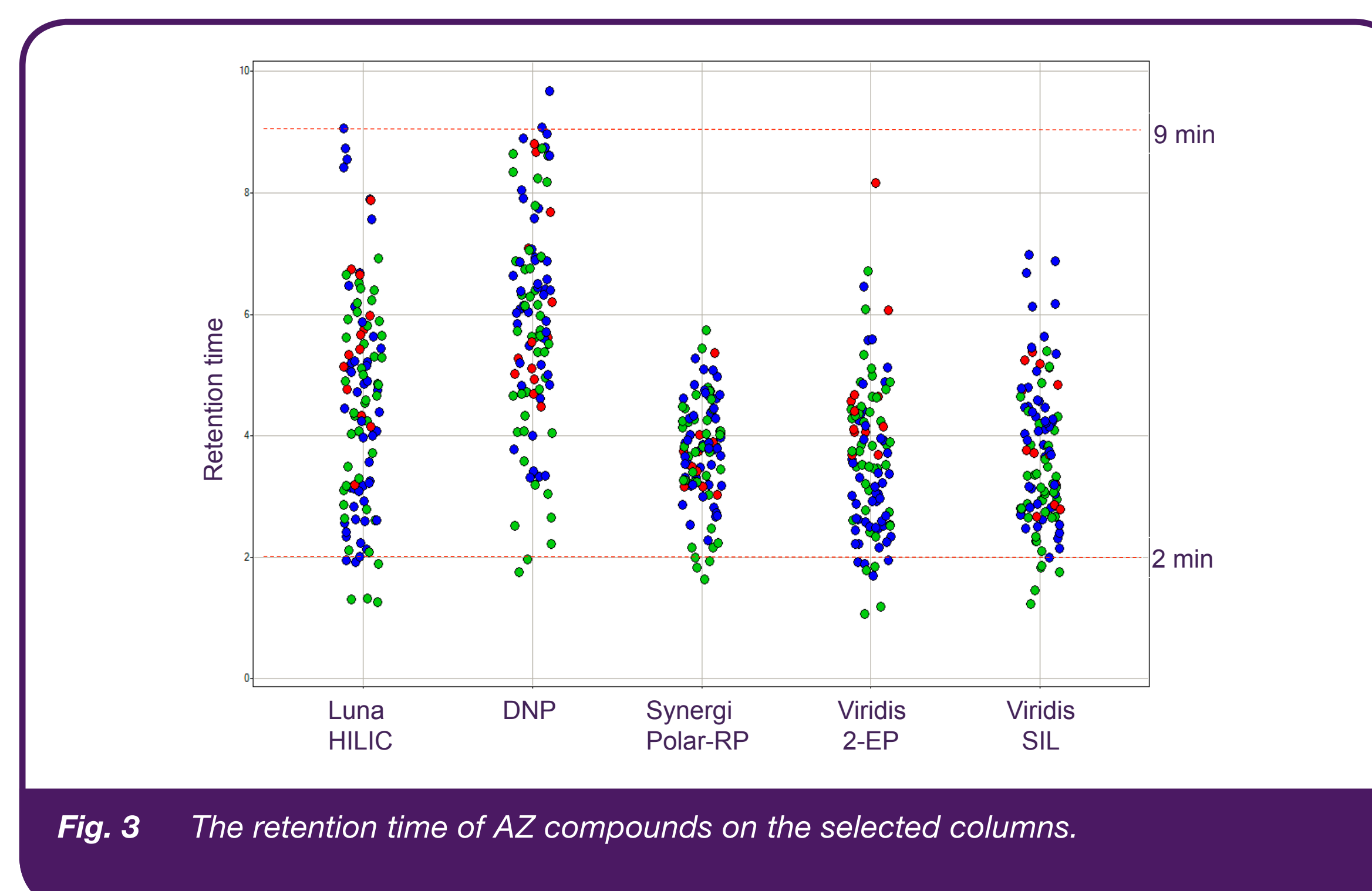
The retention of the test compounds on each column was compared to the results from the Viridis 2-EP column (Fig. 2), which earlier had been used in a demo study with good results. The screen columns were chosen to give as different selectivity as possible compared to Viridis 2-EP. The selected set of five columns is shown in bold in Table 1.



EVALUATION OF THE COLUMN SET USING CRUDE AZ COMPOUNDS

The column set was evaluated using a large set of crude AZ compounds carefully selected from the plate purification service.

The crude compounds were analyzed in single mode to make sure that the target ion could be identified on all columns. The retention times of different AZ compounds spread over the gradient and the selectivity varies between the columns (Fig. 3).

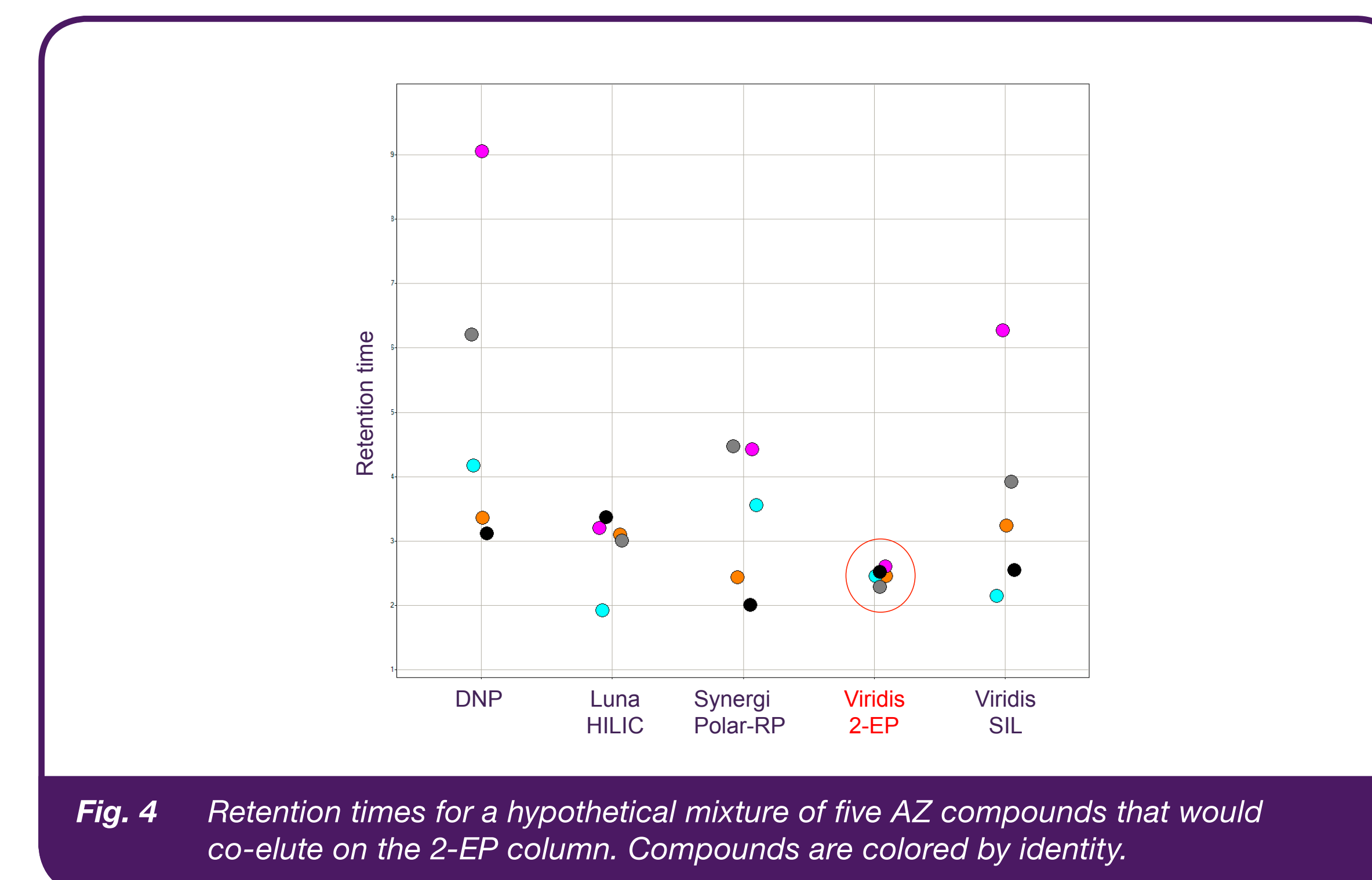


Based on visual inspection of analytical results, up to 90% of the crude AZ project compounds could have been purified with SFC using at least one of the five stationary phases in the first screen.

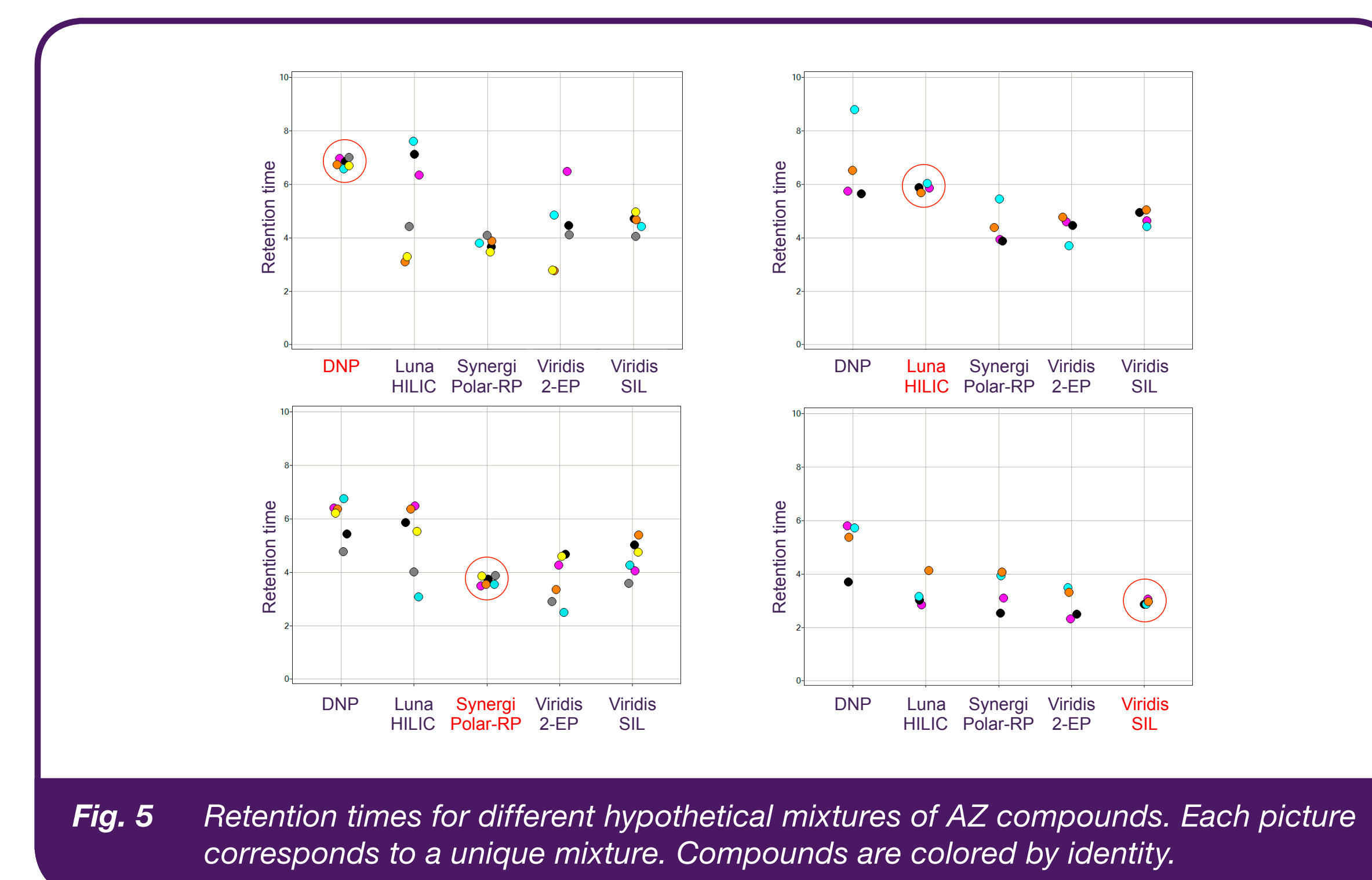
When looking only at compounds that were unsuccessfully purified with RP HPLC, it was estimated that 32 out of 39 could have been purified using SFC with this set of columns.

DIFFERENCE IN COLUMN SELECTIVITY

The difference in selectivity between the columns was further investigated using hypothetical mixtures of crude AZ compounds that had the same retention time on one of the columns. For example, five compounds with almost the same retention time on the 2-EP column would easily have been separated using the Viridis SIL column (Fig. 4).



The same evaluation was performed for all columns using different hypothetical mixtures (Fig. 5). The results indicate that most compounds could have been isolated from the mixture using at least one of the columns.



CONCLUSIONS

Out of 19 columns, five columns with different selectivity were selected for the initial screen in a plate purification service: Viridis 2-EP, Viridis SIL, Luna HILIC, Synergi Polar-RP and Princeton DNP.

Analytical results indicate that up to 90% of crude AZ compounds can successfully be purified using SFC-MS.

Based on the X5 analytical data, SFC would probably have given a better result for 32 out of 39 compounds unsuccessfully purified by RP HPLC.

ACKNOWLEDGEMENT

Stéphane Dubant, Waters
Tomas Leek and Johan Ulander, AstraZeneca

Achiral separations using a generic SFC-MS method

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SELECTING A COLUMN SET FOR SCREEN

A test set was prepared using drug-like compounds with varying properties such as size, lipophilicity and acid-base properties (Fig.1).

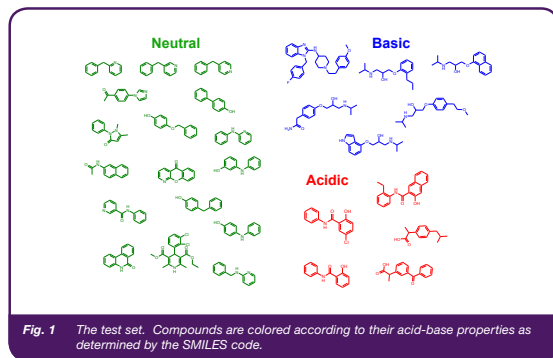


Fig. 1 The test set. Compounds are colored according to their acid-base properties as determined by the SMILES code.

The compounds in the test set were analyzed in parallel mode using the same method on 19 different stationary SFC phases (Table 1).

Waters Viridis 2-EP	Princeton 4-EP
Waters Viridis SIL	Princeton PPU
Phenomenex Luna HILIC	Princeton Pyridine Amide
Phenomenex Synergi Polar-RP	Princeton Benzamide
Princeton DNP	Princeton PA
Kromasil NH2	Princeton Benzene Sulfonamide
Kromasil SIL	Princeton 2CN-DIOL
Kromasil Diol	Princeton DEAP
Kromasil Phenyl	Princeton PPF
Kromasil CN	

Table 1 Stationary phases (column dimension: 250 x 4.6 ID mm). Selected columns are in bold.

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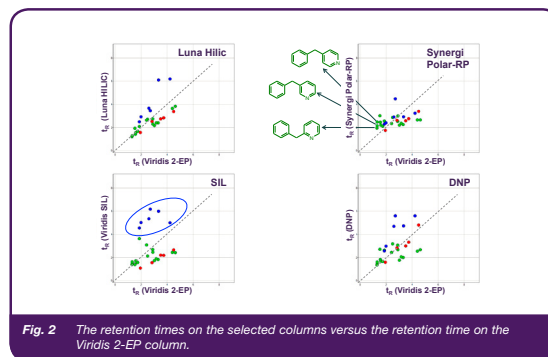


Fig. 2 The retention times on the selected columns versus the retention time on the Viridis 2-EP column.

EVALUATION OF THE COLUMN SET USING CRUDE AZ COMPOUNDS

The column set was evaluated using a large set of crude AZ compounds carefully selected from the plate purification service.

The crude compounds were analyzed in single mode to make sure that the target ion could be identified on all columns. The retention times of different AZ compounds spread over the gradient and the selectivity varies between the columns (Fig. 3).

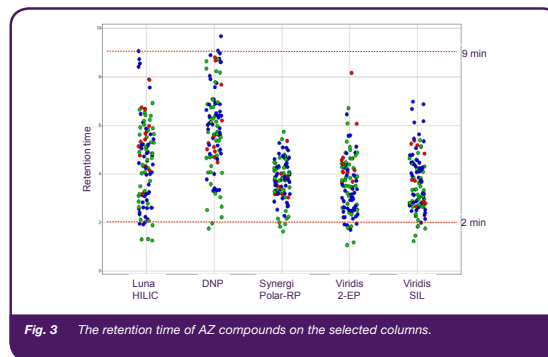


Fig. 3 The retention time of AZ compounds on the selected columns.

Based on visual inspection of analytical results, up to 90% of the crude AZ project compounds could have been purified with SFC using at least one of the five stationary phases in the first screen.

When looking only at compounds that were unsuccessfully purified with RP HPLC, it was estimated that 32 out of 39 could have been purified using SFC with this set of columns.

DIFFERENCE IN COLUMN SELECTIVITY

The difference in selectivity between the columns was further investigated using hypothetical mixtures of crude AZ compounds that had the same retention time on one of the columns. For example, five compounds with almost the same retention time on the 2-EP column would easily have been separated using the Viridis SIL column (Fig. 4).

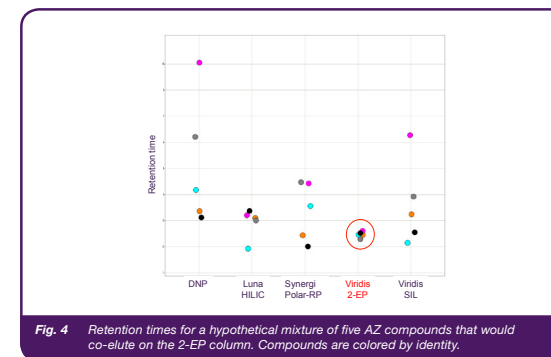


Fig. 4 Retention times for a hypothetical mixture of five AZ compounds that would co-elute on the 2-EP column. Compounds are colored by identity.

The same evaluation was performed for all columns using different hypothetical mixtures (Fig. 5). The results indicate that most compounds could have been isolated from the mixture using at least one of the columns.

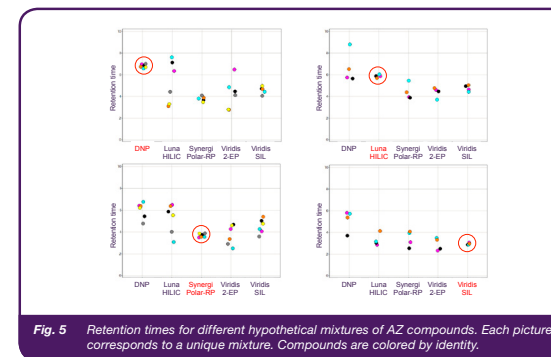


Fig. 5 Retention times for different hypothetical mixtures of AZ compounds. Each picture corresponds to a unique mixture. Compounds are colored by identity.

CONCLUSIONS

Out of 19 columns, five columns with different selectivity were selected for the initial screen in a plate purification service: Viridis 2-EP, Viridis SIL, Luna HILIC, Synergi Polar-RP and Princeton DNP.

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