Superior Peak Shape of Xanthines and Metabolites Separated by Eclipse Plus C18

Application Brief

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Food and Beverage

A new, improved HPLC column technology is shown to provide better peak shape and efficiency. ZORBAX Eclipse Plus columns combine improved ZORBAX Rx-Sil, a high-purity, type B silica that is engineered for the best peak symmetry, with optimized bonding (C18, C8) reagents and bonding processes. The result is superior peak shape and efficiency.

Xanthines are naturally occurring alkaloids, synthesized by certain species of plants. Caffeine is the most widely known, found in coffee beans, tea leaves, and cacao beans. Others found in food and beverages include theophylline and theobromine. Below is an isocratic analysis of xanthines and metabolites on Eclipse Plus and four other C18 columns. While all provide satisfactory peak shape and resolution, to the trained eye, one chromatogram exhibits exceptional chromatographic performance, Eclipse Plus C18 (PN 959993-902). For every peak in this analysis, Eclipse Plus C18 consistently has sharper peaks (higher plates (N) and better peak shape (TF)).

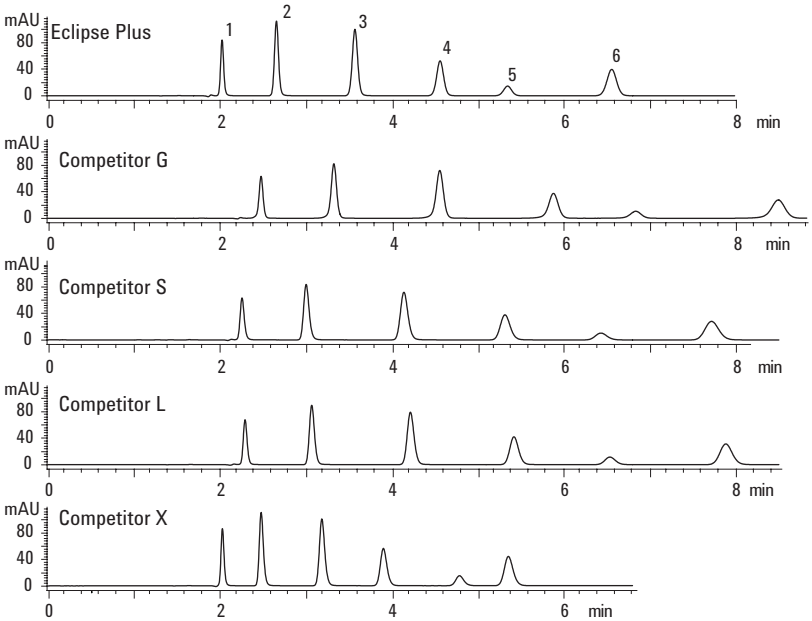

Figure 1. Separation of xanthines on various C18 columns.

Highlights

Advanced HPLC technology incorporated into Eclipse Plus columns delivers superior chromatographic performance.

Zorbax Eclipse Plus C18 columns separate xanthines with similar selectivity to other C18 columns, however with superior peak shape. Sharper peaks yield higher sensitivity.

Efficiency (N) of Eclipse Plus C18 is over 10% higher than any competitor for this analysis.

Tailing factor (TF) of Eclipse Plus C18 is closer to ideal (1.00) for every peak in this analysis compared to the competition.

Figure 1 analyte elution order:

1. 1-Methylxanthine
2. Theobromine
3. Theophylline
4. β-hydroxyethyltheophylline
5. 3-Propylxanthine
6. Caffeine

A: 25 mM Na phosphate pH 7.0
B: MeCN (90:10)
Flow: 1 mL/min
Temperature 40 °C
Columns 4.6 × 150 mm 5 µm

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Tables 1 and 2 show the difference in efficiency and peak shape between Eclipse Plus and other C18 columns based on the chromatograms in Figure 1. Clearly, Eclipse Plus C18 has higher efficiency and better peak shape than any of the competitors.

Many factors influencing efficiency and peak shape include: extra column volume, detector data sampling rate, and mobile phase conditions. These factors can be ignored in this example however, because the chromatographic system and method were the same for each analysis. Therefore, the difference in performance is entirely from the column.

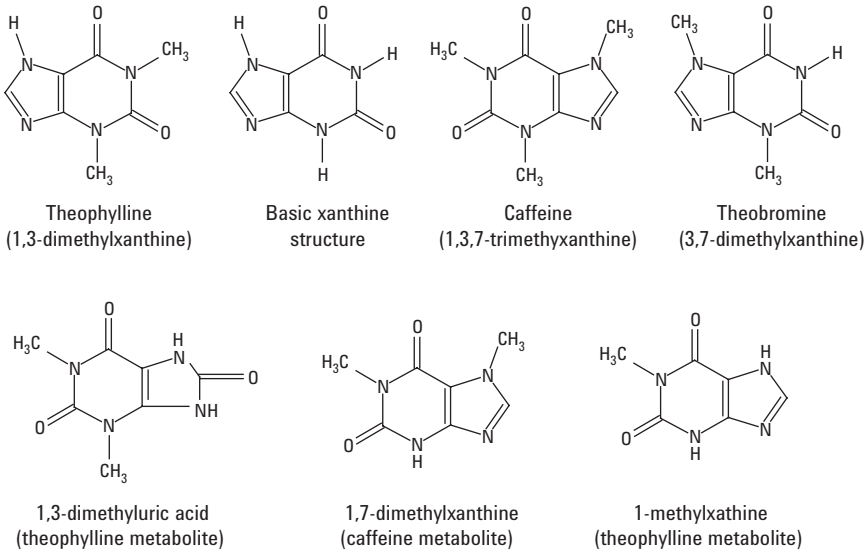
Factors attributed to the column that influence efficiency include: silanol interactions with basic analytes such as xanthines (see Figure 2 for chemical structures), column voids inherent in manufacture or from dissolution of silica from mobile phase, and particle size and distribution (column loading). Eclipse Plus' superior peak shape over the others is based on advances in HPLC technology that reduce or eliminate these factors.

Table 1: Theoretical Plates (N) of Xanthines by Various C18 Columns

	Eclipse Plus	G	S	L	X
1-Methylxanthine	14900	13100	10700	12600	15900
Theobromine	15100	13300	10700	12600	13100
Theophylline	15100	13800	10400	13400	12600
β-hydroxyethyltheophylline	15000	13300	10100	13200	12400
3-Propylxanthine	14500	13500	10400	13300	12700
Caffeine	15600	13600	10600	14000	12800

Table 2: Tailing Factor (USP 5%) of Xanthines by Various C18 Columns

	Eclipse Plus	G	S	L	X
1-Methylxanthine	1.11	0.94	1.18	1.13	1.15
Theobromine	1.08	0.95	1.19	1.14	1.18
Theophylline	1.05	0.93	1.15	1.12	1.17
β-hydroxyethyltheophylline	1.02	0.92	1.13	1.10	1.15
3-Propylxanthine	1.01	0.91	1.11	1.09	1.14
Caffeine	1.00	0.91	1.10	1.09	1.14



Theophylline (1,3-dimethylxanthine) | Basic xanthine structure | Caffeine (1,3,7-trimethyxanthine) | Theobromine (3,7-dimethylxanthine)

1,3-dimethyluric acid (theophylline metabolite) | 1,7-dimethylxanthine (caffeine metabolite) | 1-methylxathine (theophylline metabolite)

Figure 2. Structures of xanthines and metabolites in this study.

References

"High Throughput Separation of Xanthines by Rapid Resolution HPLC", Agilent publication 5989-4857EN

"New ZORBAX Eclipse Plus LC Columns", Agilent publication 5989-4934EN

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