Electronic Supporting Information

A new HPLC-UV derivatization approach for the determination of potential genotoxic benzyl halides in drug substances

Shunli Ji[a, b], Hongbin Gao[c], Xingya Xia[a, b], Feng Zheng[a, b,*]

[a]*Department of Pharmaceutical Analysis, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China*

[b]*Key Laboratory of Drug Quality Control and Pharmacovigilance, Ministry of Education, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China*

[c]*Department of Pharmacy, Baoshan Branch of Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200444, China*

**Corresponding author at: Key Laboratory of Drug Quality Control and Pharmacovigilance, Ministry of Education, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China.*

Tel.: +86 25 83271251.

E-mail addresses: cpu_analyst@126.com

Correspondence:

Dr. Feng Zheng

Department of Pharmaceutical Analysis, China Pharmaceutical University, No.24, Tongjiaxiang, Nanjing 210009, China

Email: cpu_analyst@126.com

Tel/Fax: +86 25 83271251

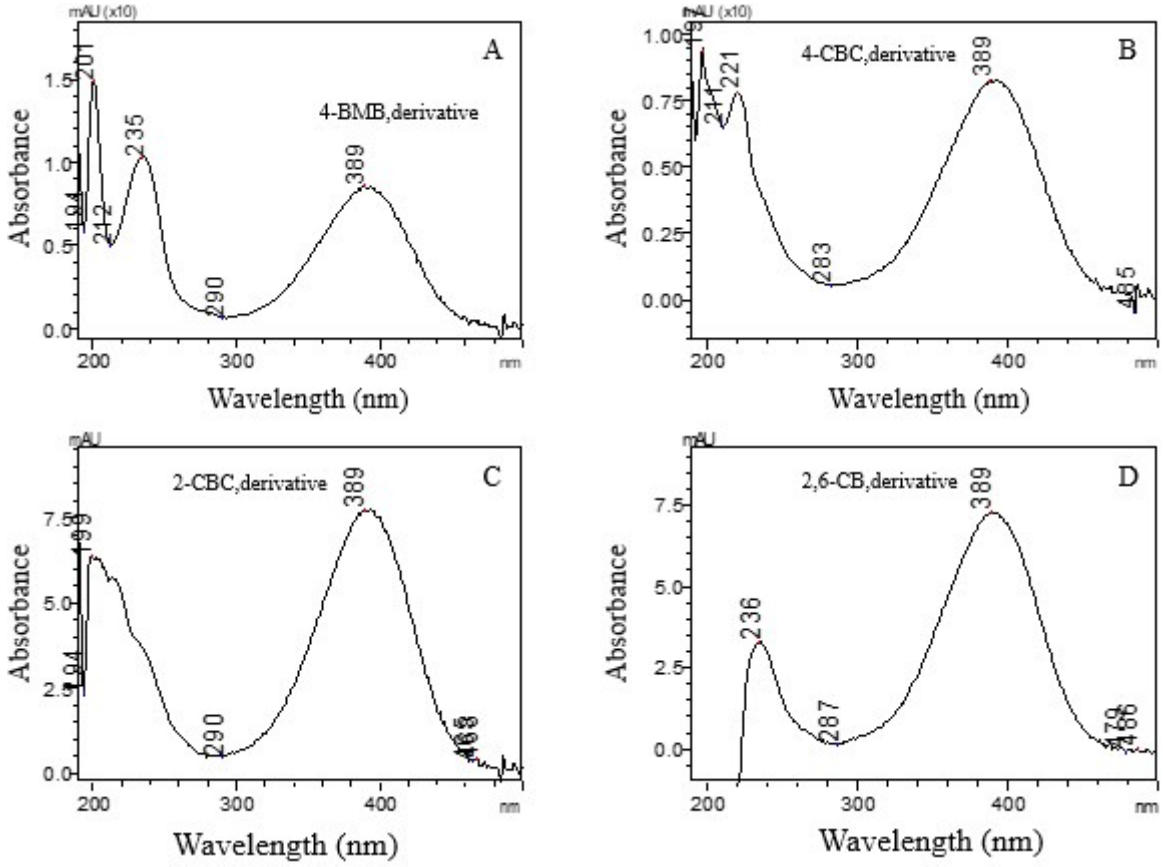

Fig. S1. UV spectrums of 4-BMB (A), 4-CBC (B), 2-CBC (C) and 2,6-CB (D) derivatives using 4-NPP as the derivatization reagents. The spiking level of 4-NPP was 300 μg·mL^{-1}. All the derivatization reactions were in a water bath of 60 °C for 60 min.

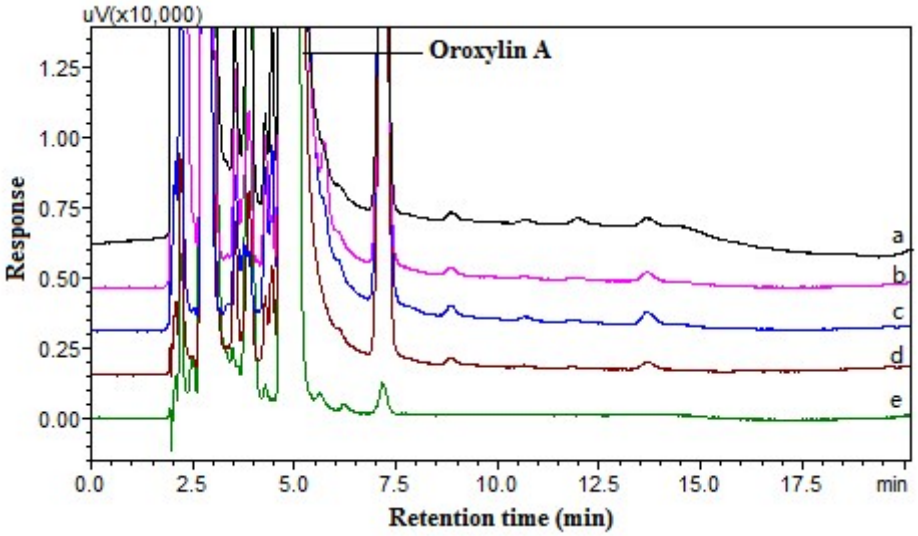

Fig. S2. Typical HPLC Chromatograms of oroxylin A after derivatization with 4-NPP at different absorption wavelengths (a. 230 nm; b. 254 nm; c. 280 nm; d. 326 nm; e. 392 nm).

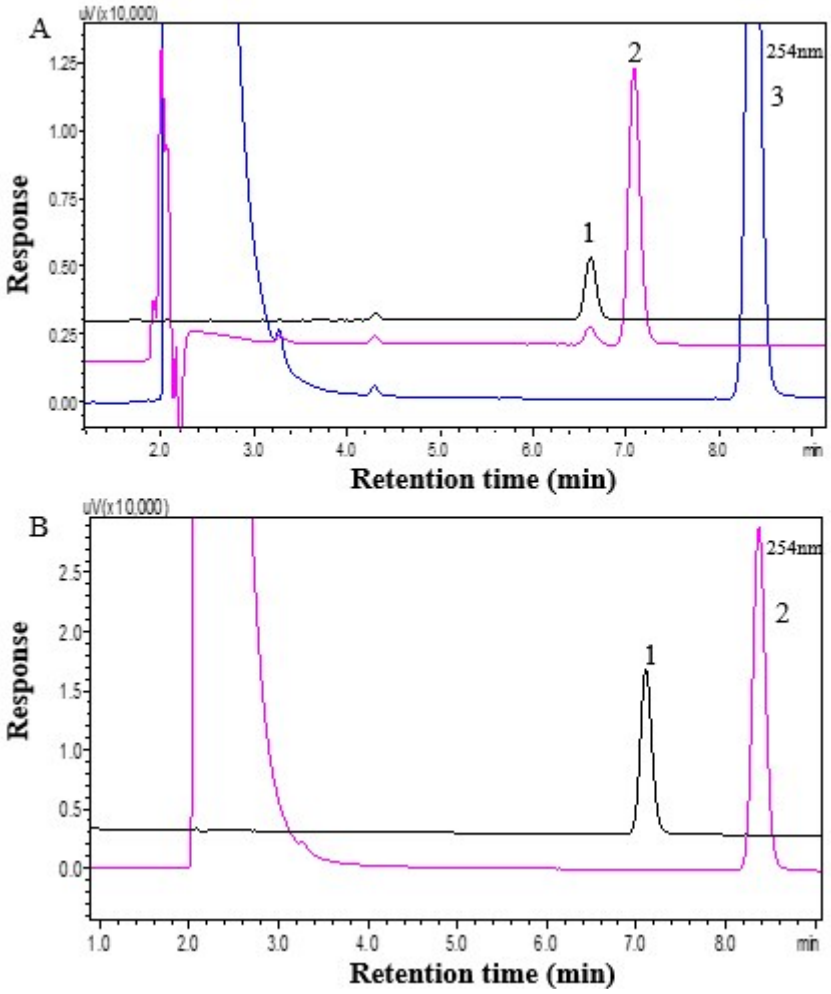

Fig. S3. (A) Typical HPLC Chromatograms of benzyl chloride (black line), benzyl chloride+KBr (pink line) and benzyl chloride+KI (blue line). Peak identification: benzyl chloride (1), benzyl bromide (2), benzyl iodide (3). (B) Typical HPLC Chromatograms of benzyl bromide (black line) and benzyl bromide+KI (pink line). Peak identification: benzyl bromide (1), benzyl iodide (2).

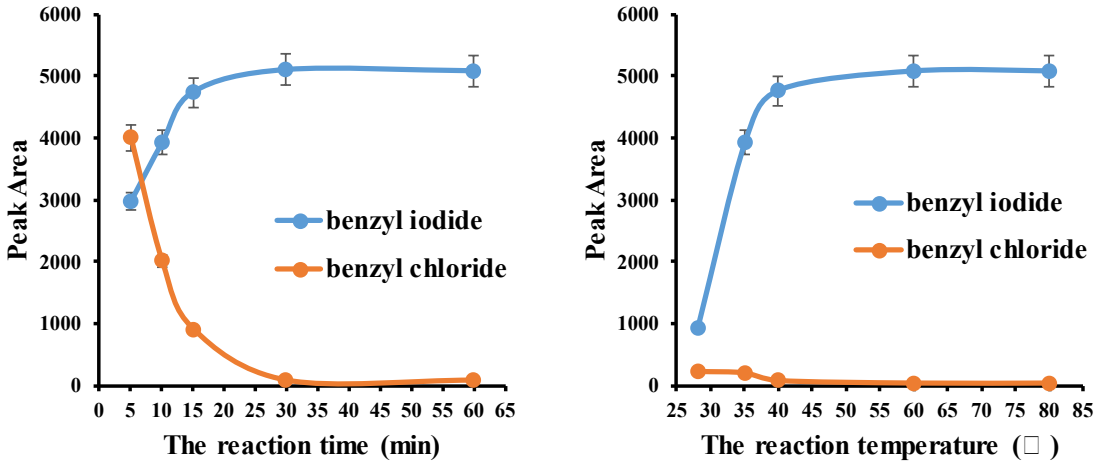

Fig. S4. Effect of reaction time (A) and reaction temperature (B) on conversion efficiency of benzyl chlorides. The concentration of KI was 40 mg·mL^{-1}.

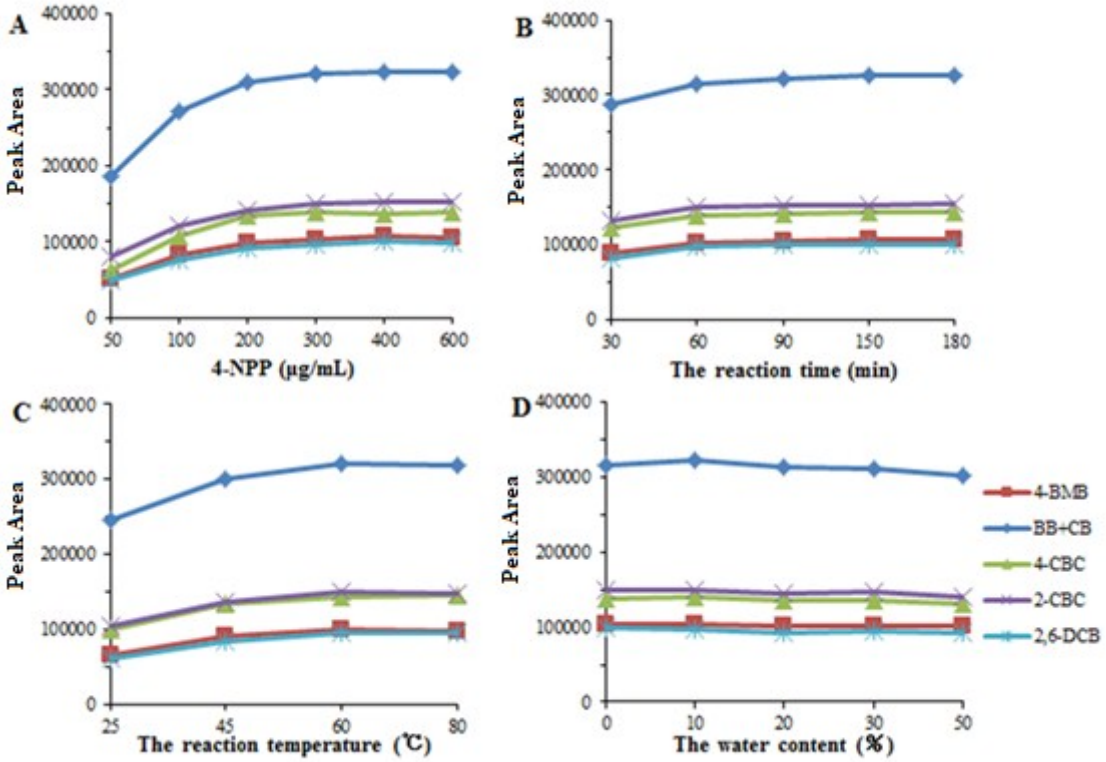

Fig. S5. Effect of the concentration of 4-NPP (A), reaction time (B), reaction temperature (C) and the water content (%, v/v) on derivatization efficiency. The concentration of each benzyl halides was 1 μg·mL^{-1}.

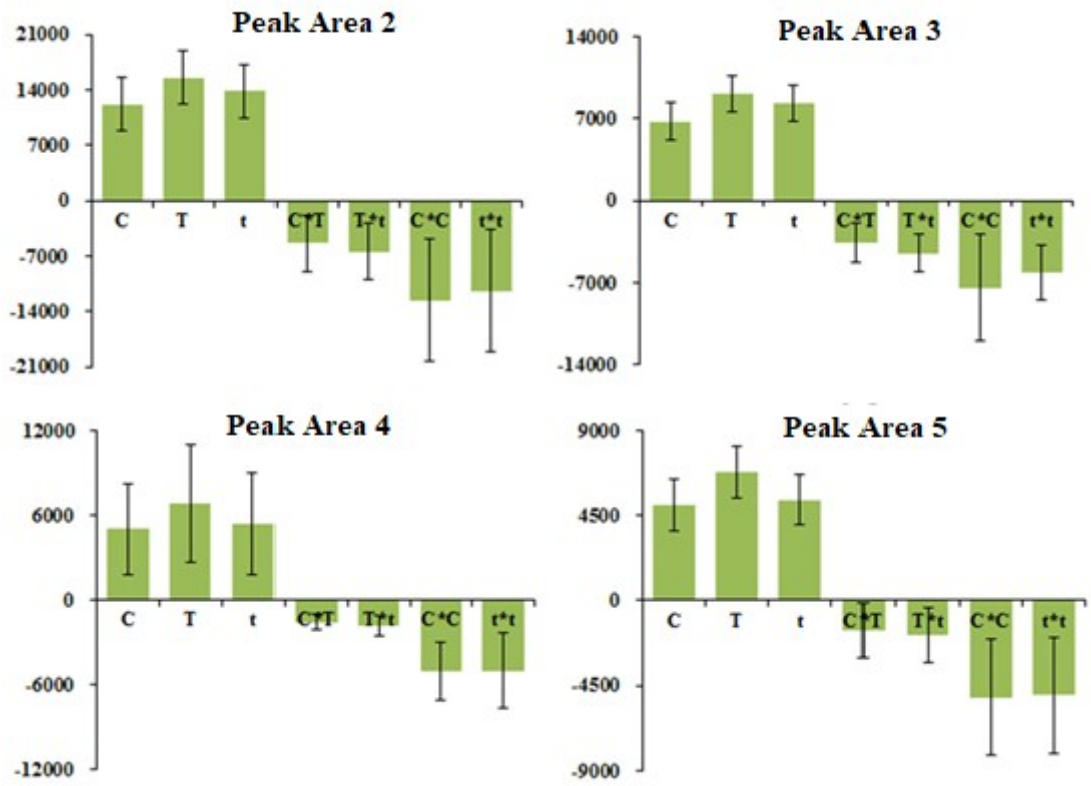

Fig. S6. Regression coefficient of the quadratic model and their confidence intervals for each response. Peak Area 2-5 represent the peak areas of BB and BC derivative, 4-CBC derivative, 2-CBC derivative and 2, 6-DCB derivative, respectively.

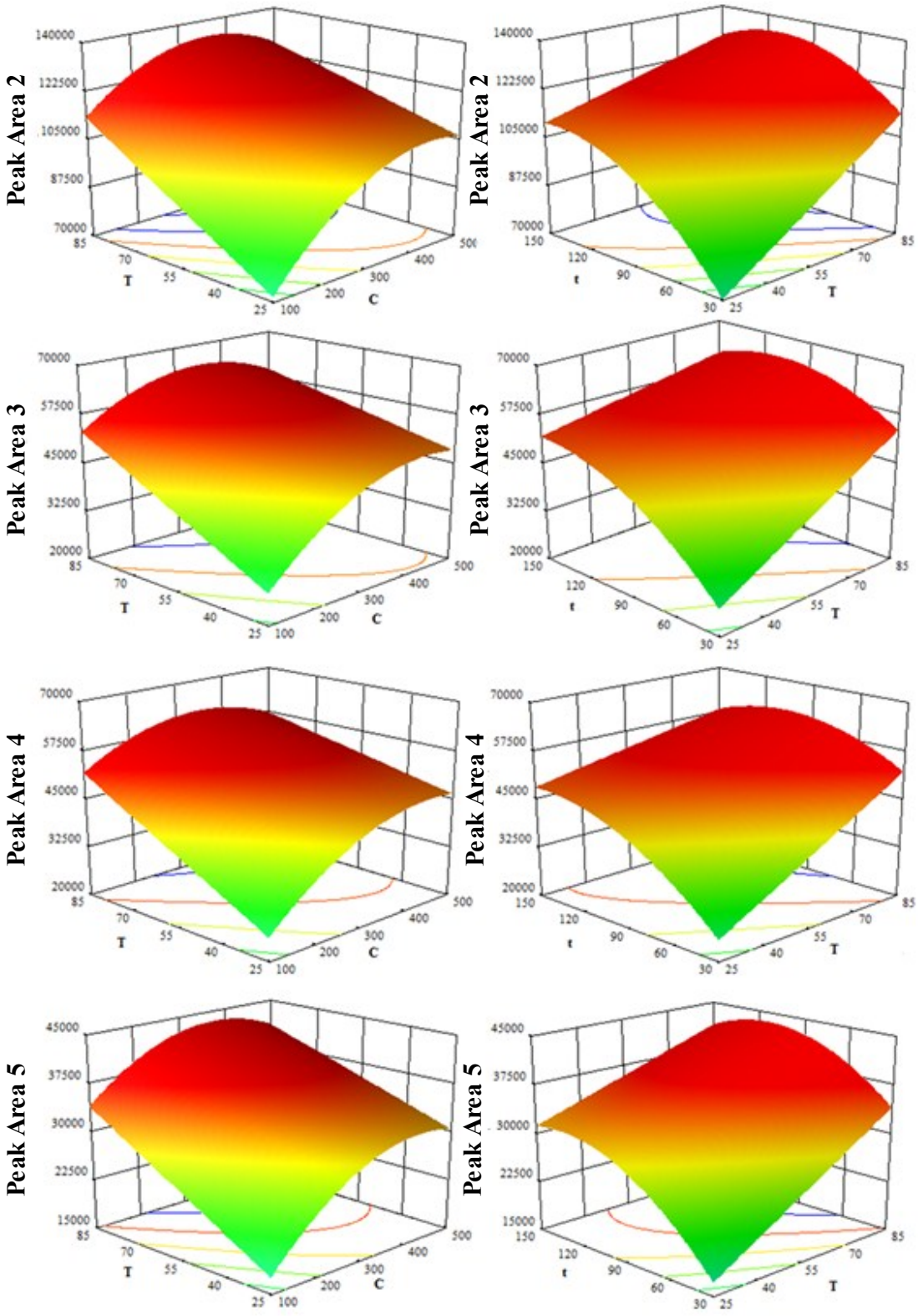

Fig. S7. Dependence of Peak Area 2-5 on C, T and t. The concentration of each analyte was 400 ng·mL^{-1}.

Table S1 Systematic comparison of existing methods with the developed method for the determination of benzyl halides in the drug substances.

Methods	LOD ($\mu g \cdot g^{-1}$)	LOQ ($\mu g \cdot g^{-1}$)	matrix	Comments
SPME GC-FID[1]	0.3	0.9	Unknown drug substance	Need SPME fibers and only suitable for the detection of volatile benzyl chloride
HS-GC-ECD[2]	0.0025-0.01	/	Warfarin / Genentech internal Compound 1	Need ionic liquids as diluents
Derivatization LC-MS/MS[3]	/	Benzyl bromide ： 1-10 Benzyl chloride： poor sensitivity due to the low derivatization efficiency	Paracetamol/caffeine	Need LTQ or TSQ MS spectrometer and a long derivatization time
Derivatization LC-MS/MS[4]	Benzyl bromide ： 1-20	/	Unknown drug substance	Need the LTQ-Orbitrap and a long derivatization time
Derivatization CE-MS[5]	Benzyl bromide ： 0.1-0.4	/	Unknown drug substance	Need CE-MS and a long derivatization time
Derivatization HPLC-UV/Vis (our method)	3-10	7-22.5	Antipyrine/ Oroxylin A	Using simple solvents, reagents and apparatus, 1.5 h for reaction at 60 °C;

Table S2 The detailed experimental data of central composite face (CCF) DoEs.

RUN	factor 1（C）	factor 2（T）	factor 3（t）	Apeak1	Apeak2	Apeak3	Apeak4	Apeak5
1	100	25	30	13716	71235	23415	22154	17320
2	500	25	30	32975	105680	46512	45423	35241
3	100	85	30	31474	114582	51203	50136	35237
4	500	85	30	41520	123491	59465	58602	44295
5	100	25	150	14028	79543	26850	25496	18028
6	500	25	150	35102	100125	50471	48700	39584
7	100	85	150	34125	111540	52668	51602	34406
8	500	85	150	41328	127856	57409	55778	43989
9	100	55	90	23530	93748	38843	36815	26450
10	500	55	90	34213	117898	50663	48836	38594
11	300	25	90	29786	95746	39425	37850	32414
12	300	85	90	41256	120485	57140	55165	44520
13	300	55	30	36125	110657	57512	56078	40248
14	300	55	150	38549	109773	55893	54301	42186
15	300	55	90	40973	127143	59760	58789	45082
16	300	55	90	41207	126919	60153	59153	45411
17	300	55	90	40851	126640	60289	59019	45050
18	300	55	90	41459	126875	59481	59325	45571
19	300	55	90	40992	126723	60852	58938	45802
20	300	55	90	41378	127864	59479	59157	45652

References

1. R. P. Frost, M. S. Hussain and A. R. Raghani, *Journal of Separation Science*, 2003, **26**, 1097-1103.
2. T. D. Ho, P. M. Yehl, N. P. Chetwyn, J. Wang, J. L. Anderson and Q. Zhong, *Journal of Chromatography A*, 2014, **1361**, 217-228.
3. A. M. Wijk, Van, B. Beerman, H. A. G. Niederländer, A. H. G. Siebum and G. J. Jong, De, *Analytical & Bioanalytical Chemistry*, 2011, **400**, 1375-1385.
4. A. M. V. Wijk, H. A. G. Niederländer, A. H. G. Siebum, M. A. T. Vervaart and G. J. D. Jong, *J Pharm Biomed Anal*, 2013, **74**, 133-140.
5. A. M. Wijk, Van, H. A. G. Niederländer, M. D. Ogten, Van and G. J. Jong, De, *Analytica Chimica Acta*, 2015, **874**, 75-83.