Evaluation of gastrointestinal stromal tumour sample preparation procedure for LC-MS untargeted metabolomic analysis

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INTRODUCTION

In analytical method validation process, robustness is the ability of an analytical procedure to achieve undeniable results despite introducing minor changes in experimental conditions. Most often, the Plackett-Burman experimental plan is used, which reduces the number of measurements and shortens the research time. The Design of Experiments (DoE) assumes the change of many factors over time, which enables to assess the impact of each factor on the obtained results.

Quality assurance is essential in metabolomic analysis to ensure that the acquired data are of high quality. In the present study, we used the methodology of robustness testing to evaluate the sample preparation procedure of a gastrointestinal stromal tumour for metabolomic analyses with the use of HPLC-TOF/MS. Tissue is a particularly challenging type of biological matrix in metabolomic analysis due to complex sampling procedure, normalization, homogenisation, and metabolite extraction. The goal of this study was to determine the critical stages of sample preparation method, that need to be controlled in order to obtain undeniable results.

MATERIALS & METHODS

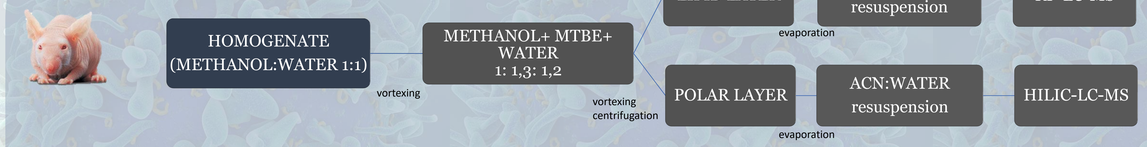

Fig. 1. Assessed GIST tissue sample preparation procedure

Table 1. Factors taken into account in Plackett-Burman experimental plan

Factor	-1	0	1
1. Methanol volume for extraction	98 µl	100 µl	102 µl
2. MTBE volume 1	196 µl	200 µl	204 µl
3. Vortex time 1	2:45 min	3 min	3:15 min
4. MTBE volume 2	315 µl	320 µl	325 µl
5. Water volume	226 µl	230 µl	234 µl
6. Vortex time 2	0:50 min	1 min	1:10 min
7. Centrifugation time(18000G)	9 min	10 min	11 min
8. Volume for evaporation	196 µl	200 µl	204 µl
9. Evaporation temperature	33°C	35°C	37°C
10. Volume of solvent for resuspension	196 µl	200 µl	204 µl

Table 2. Plackett-Burman matrix for evaluating the impact of ten factors on the robustness of tumour sample preparation procedure

	wzór	methanol [µl]	MTBE1 [µl]	vortex1 [min]	MTBE2 [µl]	H20 [µl]	vortex2 [min]	centrifugation [min]	volume for evaporation[µl]	evaporation temperaute [ºC]	volume of solvent [µl]	dummy variable
1	+−−+−+−++	102	196	165	315	234	50	9	204	33	204	1
2	+++−−−+−+−	102	204	195	315	226	50	11	196	33	204	-1
3	−−+−+++−−+	98	196	195	315	234	70	11	196	33	196	1
4	−+−+−+++−−	98	204	165	315	234	50	11	204	37	196	-1
5	−−−+−−+−+++	98	196	165	325	226	50	11	196	37	204	1
6	0	100	200	180	320	230	60	10	200	35	200	0
7	+−+++−−−+−−	102	196	195	325	234	50	9	196	37	196	-1
8	−+++−−+−−+	98	204	195	325	226	50	9	204	33	196	1
9	−−+−+−+++−	98	196	195	315	226	70	9	204	37	204	-1
10	0	100	200	180	320	230	60	10	200	35	200	0
11	++−−+−−−+−	102	204	165	315	226	70	9	196	37	196	1
12	++++++++++	102	204	195	325	234	70	11	204	37	204	1
13	+−−+−+++−−	102	196	165	325	226	70	11	204	33	196	-1
14	−+−+++−−+−	98	204	165	325	234	70	9	196	33	204	-1
15	0	100	200	180	320	230	60	10	200	35	200	0

Selected responses related with robustness:

1) First principal component
2) Second principal component
3) Sum of signal intentities

RESULTS

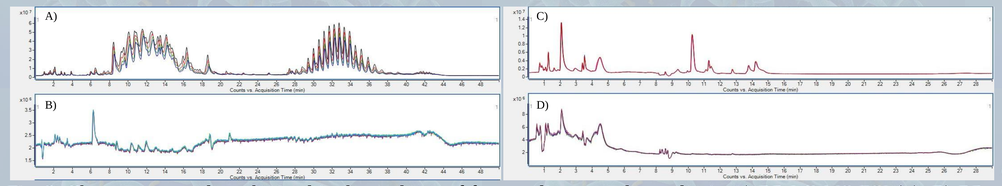

Fig. 2. Chromatogram plots obtained in the analyses of four quality control samples in A) RP-LC-MS ESI (+), B) RP-LC-MS ESI (-), C) HILIC-LC-MS ESI (+), D) HILIC-LC-MS ESI (-)

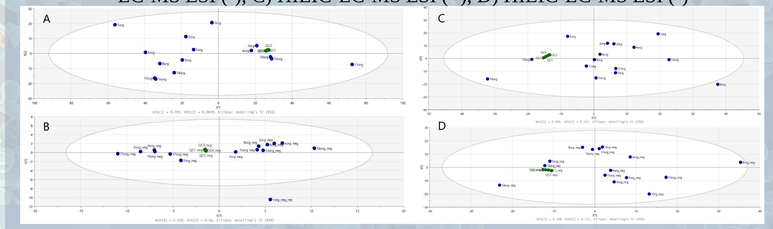

Fig. 3. PCA models built on data collected with different analytical techniques: A) RP-LC-MS ESI(+), B) RP-LC-MS ESI (-), C) HILIC-LC-MS ESI (+), D) HILIC-LC-MS ESI (-). Green dots correspond to quality controls, while blue dots refer to experimental samples

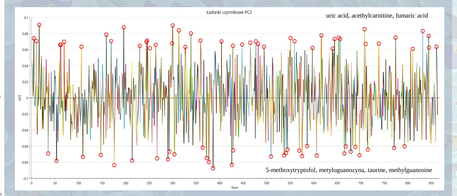

Fig. 4. PCA loading values of second principal component in PCA analysis of data obtained with HILIC-LC-MS ESI(-)

Table. 3. Impact of all examined factors on three selected responses regarding the extraction process of metabolites determined by means of HILIC-LC-MS ESI(-)

	PC1		PC2		Sum of signals	
Factor	Regression coefficient	p value	Regression coefficient	p value	Regression coefficient	p value
MTBE1 [µl]	0,43826	0,9205	1,63328	0,2938	-783297	0,6570
Evaporation temperature [°C]	-4,31644	0,2792	**9,43614**	**0,0020***	-221548	0,8997
MTBE2 [µl]	-0,92217	0,8338	1,3428	0,3849	1253712	0,4063
Vortex2 [min]	-4,40753	0,2711	0,52140	0,7701	-1032880	0,5399
Solvent volume [µl]	-6,36121	0,1237	-2,29124	0,1586	-1143834	0,4573
Methanol [µl]	2,15001	0,6381	0,07094	0,9688	85293	0,9625
Volume for evaporation [µl]	6,66980	0,1106	0,78559	0,6634	227527	0,8968
Dummy factor	2,50234	0,5819	-0,35692	0,8431	935334	0,5924
Centrifugation [min]	0,10300	0,9803	-1,10296	0,5081	1165660	0,4458
Vortex1 [min]	2,96961	0,4641	3,24533	0,0630	1137943	0,4599
H20 [µl]	-0,18321	0,4267	-2,23084	0,1676	-1563081	0,3019

CONCLUSIONS

1) The study shows that the critical stage of tissue sample extraction procedure is the temperature of solvent evaporation in the centrifugal concentrator. Instability of evaporation temperature proved to impact the value of the second principal component in the PCA model built on LC-MS data collected during HILIC analysis, in negative ionisation mode. For data obtained from RP-LC-MS or HILIC-LC-MS ESI(-) analyses, none of the factors was significantly affecting selected responses.
2) Methylguanosine, acetylcarnitine, or fumaric acid were the most sensitive to fluctuations in evaporation temperature.
3) According to the research results, strict control of the critical factor should be performed. The condition of the centrifugal concentrator used during the extraction procedure should be verified before each project to ensure correct vacuum and temperature levels in the device.

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