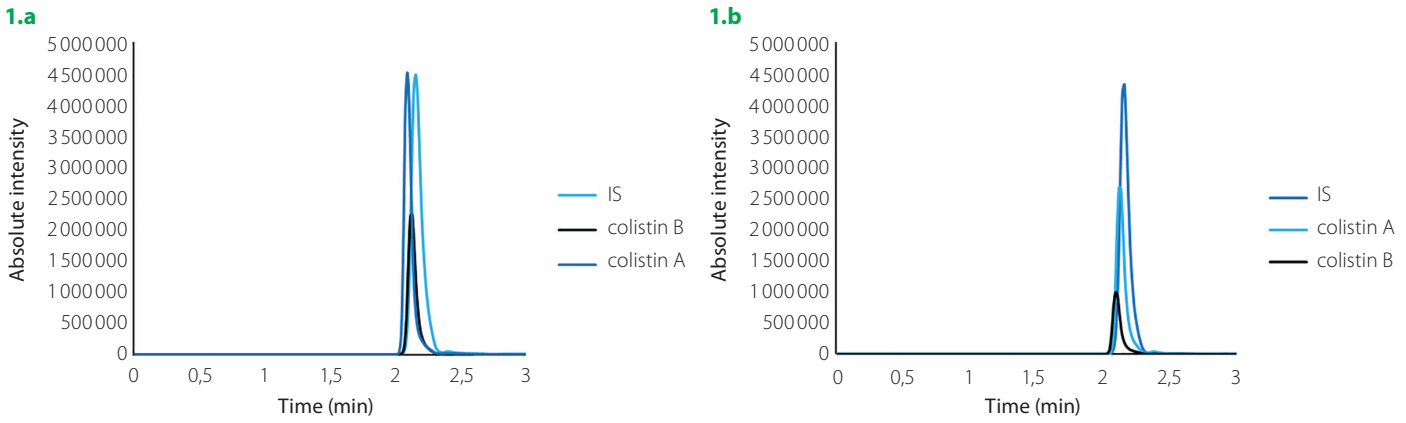
Fig. 1. *Representative chromatograms of calibration point 10 mg/L (1.a) and patient sample (1.b)*



of R^2 = 0,9995 for colistin A and R^2 = 0,9982 for colistin B. The results of the inter-day and intraday measurements for method validation are accurate and precise, with an error not exceeding 15 % with the LOQ set at 0,15 mg/L. The validation confirmed the reliability of the LC-MS method for measuring concentrations of colistin in human plasma. Summary information on the analysis parameters is given in Table 1.

The CMS concentration was measured indirectly by acid hydrolysis, for which we found it most useful to use 15 µl of 1 M sulfuric acid. After 30 minutes, the hydrolysis was stopped by adding 30 µl of 1M sodium hydroxide. As this is an indirect method, it is necessary to back-calculate the CMS concentrations from the difference:

$$CMS = COL_{total} - COL_{before\ hydrolysis}$$

where COL_{total} is the concentration after hydrolysis of CMS to colistin and $COL_{before\ hydrolysis}$ is the circulating concentration of colistin formed by endogenous transformation of the prodrug to its active form.

Long-term stability studies of colistin showed no degradation in stock solutions and patient plasma samples stored at –70 °C for at least 90 days. Also, no degradation was observed in three freeze-thaw cycles (data not shown). The short-term stability of colistin was measured for three concentration points at room temperature (RT) and 37 °C (Table 2). After 24 hours at RT, the degradation of the samples reached almost 10 %. However, the degradation of colistin was more significant at 37 °C. Already after 30 minutes, degradation reaching up to 25 % was observed, the average degradation for all samples was 11 %.

Tab. 1. *Summary information on the analysis parameters*

Chromatographic conditions		
HPLC:	Shimadzu, Prominence LC–20A	
Column:	Arion® Polar C18 (250 × 4,6 mm; 5 mm)	
Mobile phase A: Mobile phase B:	0,1% formic acid in water 0,1% formic acid in methanol	(40:60, v/v)
Flow rate:	0,8 ml/minute	
Column temperature:	35 °C	
The volume of injection:	10 µl	
Analysis time:	3 minutes	
MS/MS detection		
Mass spectrometer:	Shimadzu, LCMS-8045	
Ionization mode:	ESI positive	
Ion transition monitored:	Colistin A 585,55 → 101,05	
	Colistin B 578,5 → 101,15	
	IS 602,4 → 101,1; 120,15; 86,15	
Validation parameters		
Calibration curve range (mg/l)	0,15–30	
Limit of quantification – LOQ (mg/l)	0,2	
Limit of detection – LOD (µg/l)	4,7	
Recovery (%)	81	

Tab. 2. *Stability of colistin A and B (%) at three concentration levels (2; 10; 20 mg/L) in human plasma*

		Colistin A			Colistin B		
	Time	**2 mg/L**	**10 mg/L**	**20 mg/L**	**2 mg/L**	**10 mg/L**	**20 mg/L**
RT	0,5 h	99,28	101,28	100,18	107,01	105,14	103,41
	1 h	98,90	97,86	99,05	92,93	97,28	104,80
	2 h	96,92	97,66	96,08	92,15	95,31	99,89
	5 h	93,28	92,16	95,64	92,22	94,15	96,57
	24 h	90,57	92,71	91,37	90,15	93,93	96,46
37 °C	30 min	91,65	92,76	91,21	74,96	92,76	92,17

RT – room temperature

Tab. 3. *Stability of CMS (%) at 10 mg/L concentration level in human plasma*

	37 °C		**RT °C**	
Time	COL A	COL B	COL A	COL B
0,5 h	**103,7**	**103,1**	**100,1**	**100,6**
1 h	**108,0**	**109,2**	**102,0**	**105,6**
2 h	**114,0**	**116,3**	**108,1**	**110,3**
3 h	**117,6**	**119,9**	**111,7**	**113,8**

RT – room temperature

CMS stability in plasma samples at a selected concentration of 10mg/L was studied at room temperature and 37 °C. As expected, the conversion of CMS to colistin is more significant at 37 °C than at RT. At elevated temperatures, we observe the conversion of CMS into its colistin