

C. LE GOFF (1), S. PEETERS (1), M. NETCHACOVITCH (1), JF. KAUX (2), Y. CRINE (1), E. CAVALIER (1)

(1) Department of Clinical Chemistry, University of Liège, CHU Sart-Tilman, B-4000 Liège, Belgium.

(2) Department of motricity sciences, University of Liège, CHU Sart-Tilman, B-4000 Liège, Belgium.

Email: c.legoff@chu.ulg.ac.be

Introduction: The aim of this work was to develop and validate a method for the determination of metanephrine (M), normetanephrine (NM) and methoxymetanephrine (METHO) in urine by liquid chromatography-tandem mass spectrometry (LCMS-MS) on the Triple Quad TQ 5500 from AB SCIEX (Figure 1). In fact, the determination of M and NM concentrations is used in clinical diagnosis of pheochromocytoma, a rare but potentially fatal tumor arising primarily from the chromaffin cells of the adrenal medulla.

Materials and Methods: The samples were made of 24 hours acidified urines after centrifugation. Sample preparation was performed by hydrolysing and purifying by extraction column. After that, labeled M, NM and METHO were added as internal standard. Samples were analysed by liquid chromatography-electrospray tandem mass spectrometry. We determined the repeatability, reproducibility, accuracy profile and recovery on pooling urines samples from 9 volunteers analysed in triple run.

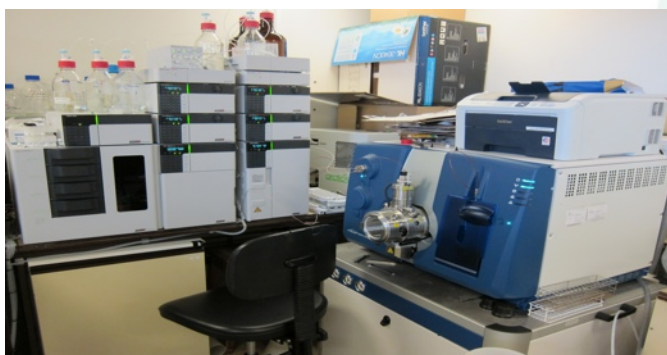


Figure 1

Results: The results of the precision evaluation are shown in table 1. The repeatability did not exceed 8.4 % for M, 6.8% for NM and 10.8% for METHO. The concentration range was 71-781 µg/24h, 71-853 µg/24h and 20-854 µg/24h for the M, NM and METHO respectively. The total precision did not exceed 12.5%, 11.8% and 8.8% for M, NM and METHO. The limit of quantification (LOQ) were 15 µg/24h, 14.49 µg/24h and 19.81 µg/24h for M, NM and METHO respectively. The accuracy varied from 99.69 to 100.2% for a range of 71 to 781 µg/24h, from 93.32 to 100.2% for a range of 71-853 µg/24h and from 99.85 to 100.6% for the range 20-854 µg/24h for M, NM and METHO respectively (Figure 2-4). The recovery were 99.96% (95% CI for the mean: 96.5-103.4), 99.75% (96.5-102.9) and 100.08 (95.97-104.2) for the M, NM and METHO respectively.

Pool META	n	Mean (µg/24h)	Within run SD	CV (%)	Total SD	CV (%)
6	9	70.78	2.848	4.011	8.433	12.54
7	9	69.11	4.702	6.815	5.568	10.56
8	9	132.7	2.809	2.11	11.28	8.737
9	9	199.3	16.66	8.37	9.709	9.69
10	9	217	5.802	2.67	10.45	5.506
11	9	781	32.62	4.17	38.03	6.415
Pool NORME	n	Mean (µg/24h)	Within run SD	CV (%)	Total SD	CV (%)
6	9	71.11	2.285	3.219	5.15	7.935
7	9	85.89	5.185	6.03	8.677	11.75
8	9	204.8	16.7	8.148	13.16	10.37
9	9	280.7	9.866	3.511	16.85	6.948
10	9	419.1	15	3.579	24.66	6.888
11	9	858.3	36.99	4.311	0.6086	4.312
Pool METHO	n	Mean (µg/24h)	Within run SD	CV (%)	Total SD	CV (%)
6	9	20.11	0.5774	10.84	2.091	2.887
7	9	71.89	3.215	7.738	4.55	4.465
8	9	208.8	18.39	10.19	10.76	8.798
9	9	854	32.61	5.291	31.27	3.819

Table 1

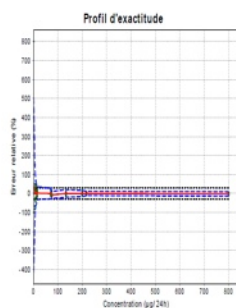


Figure 2

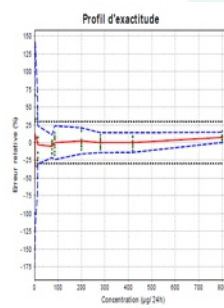


Figure 3

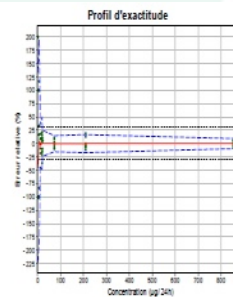


Figure 4

Conclusions: We have successfully developed and validated an LCMS-MS method to determine urinary M, NM and METHO. It represents a convincing alternative to the HPLC method for a faster and reliable measurement of urinary M and NM. and METHO.