RAPID ANALYSIS OF L-ARGININOSUCCINIC ACID (ASA) BY USING A SHORT PROGRAM OF TOTAL HOMOCYSTEINE

Tijen Tanyalcin
Tanyalcin Medical Laboratory Selective Screening and Metabolism Unit, Izmir, Turkey

AIM :This study presents a rapid quantification of ASA by the modified method of total homocysteine program for the Norleucine users as an internal standard for full - standard amioacid chromatogram on Biochrom 30 aminoacid analyser.

HISTORY : The plasma sample of argininosuccinic aciduria diagnosed case by using Aminoethyl Cysteine (AEC) as an internal standard was additionally analysed by total homocyteine program; and coincidentally; the ASA peak was seen just before Norvaline; the internal standard used for homocysteine program. Figure 1.
.Full aminoacid program chromatogram of ASA case is also seen in Figure 2

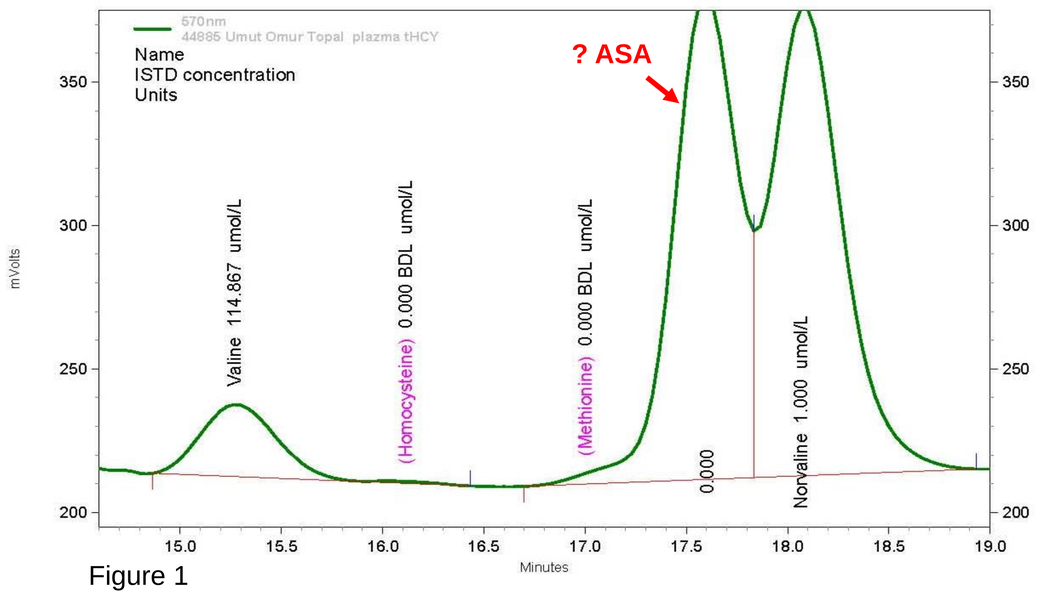


Figure 1

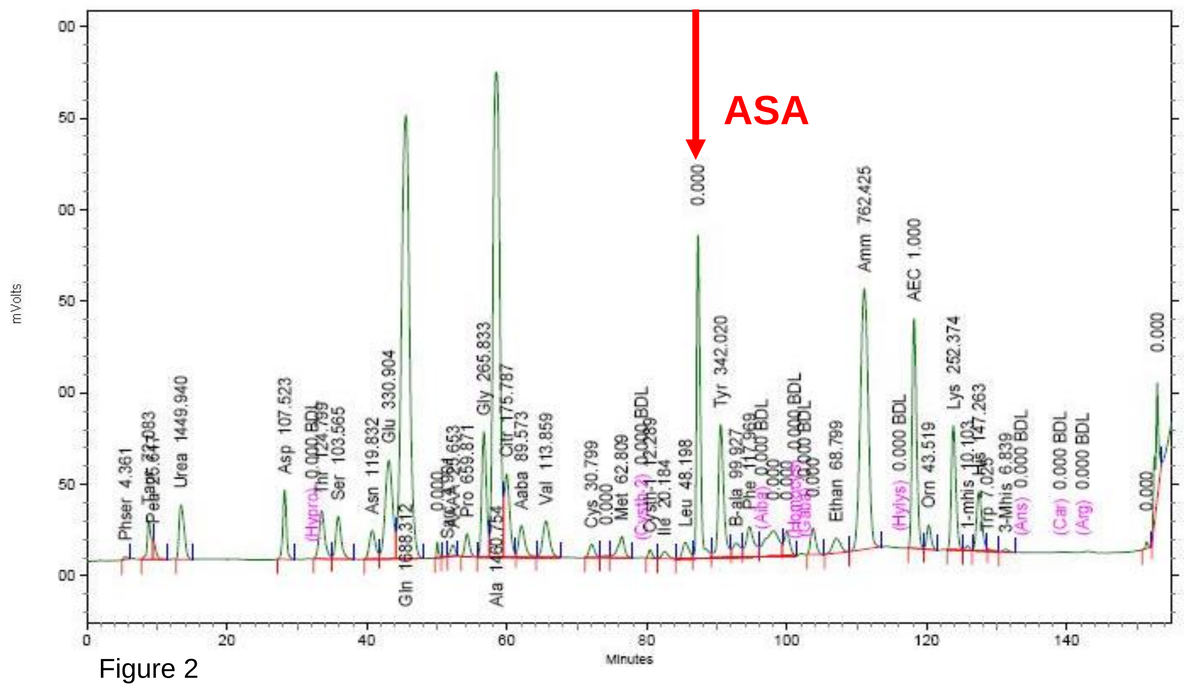


Figure 2

METHOD & WORKING PLAN The separation is achieved with 20 X 4.6 mm physiological high resolution column using predominantly buffer CII = BUFFER 3 (pH: 3.15). The rapid analysis of total homocystein program allows the ASA peak to be easily seen just before norvaline internal standard and the quantification can be made less than 30 minutes.

Reps	Sample ID
1	LB
1	ASA _500 uM_LB
1	ASA _1000 uM_LB
1	ASA _500 uM_SSA Norvalin
1	ASA _1000 uM_SSA Norvalin
1	A1_A2_ASA 500 uM_HCY 500 uM_LB WSTD
1	A1_A2_ASA 1000 uM_HCY 500 uM_LB WSTD

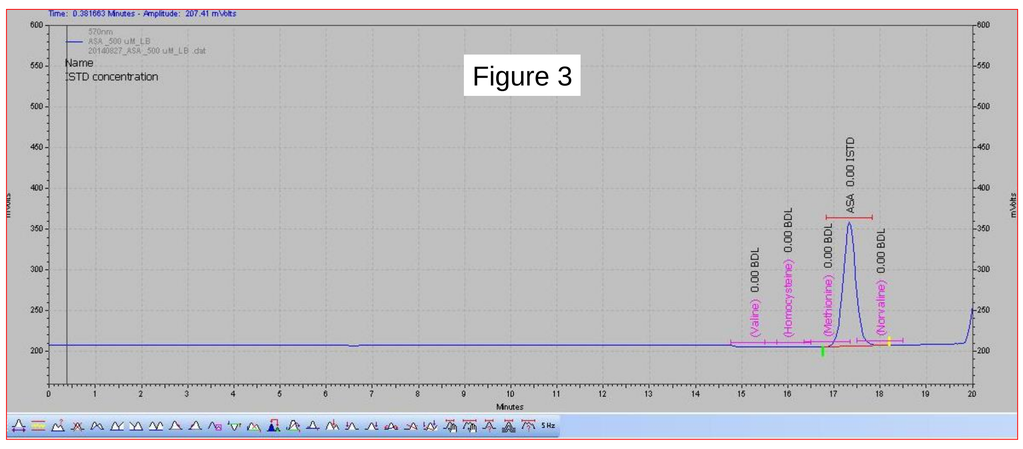


Figure 3

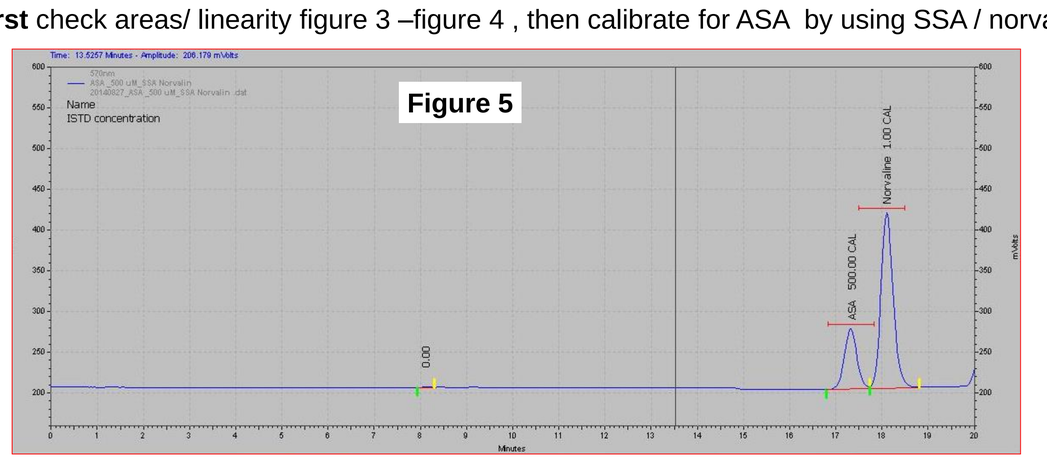


Figure 5

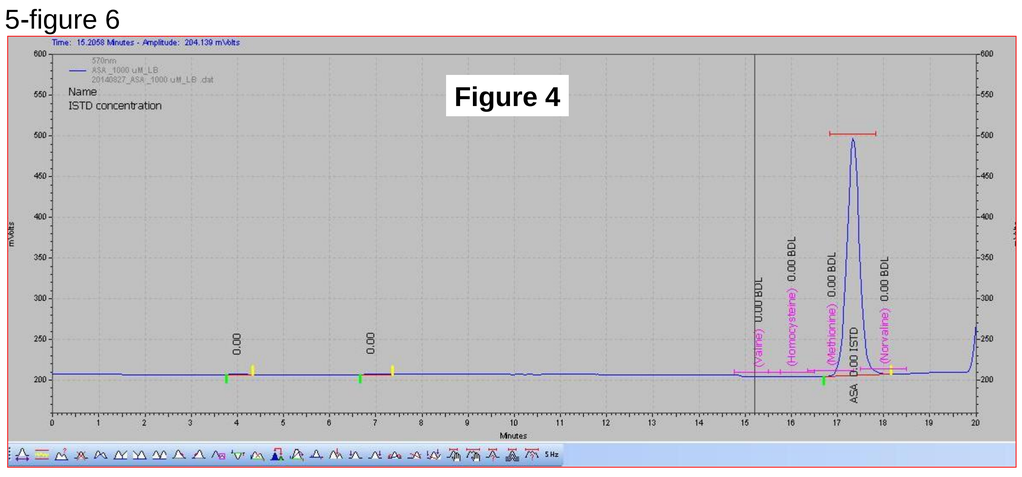


Figure 4

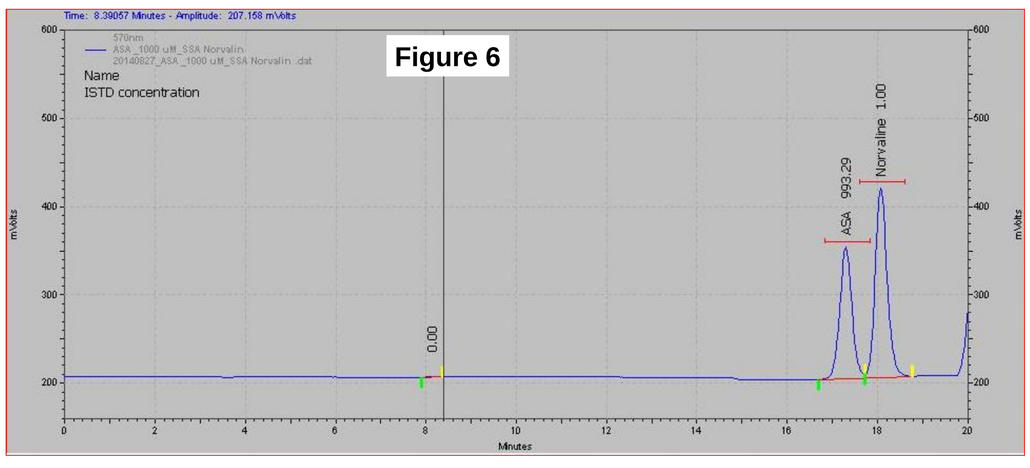


Figure 6

Second step is to see if methionine and ASA coleute ; 500 uM Methionine is used only in SSA /Norvaline IS

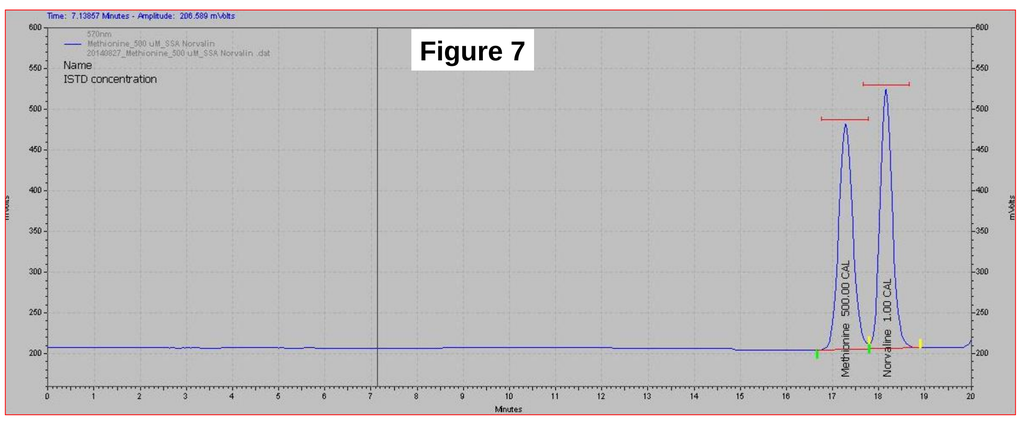


Figure 7

Third step :Freshly dissolved ASA (500 uM) analyzed with SSA/AEC

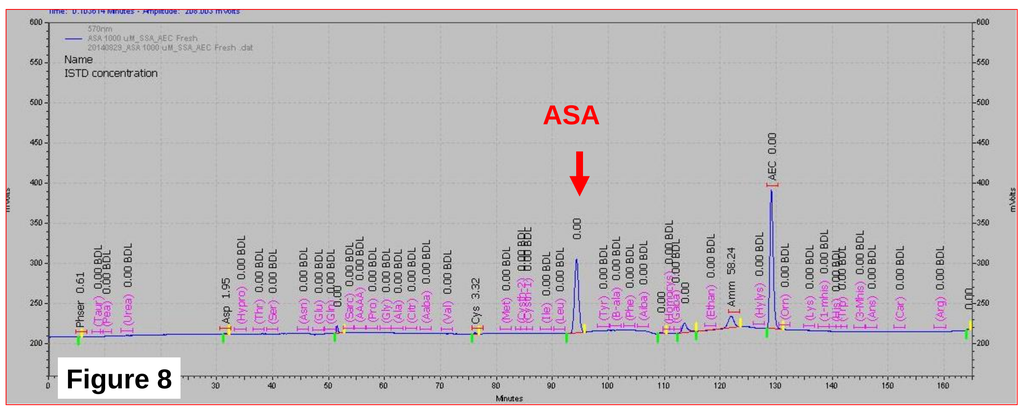


Figure 8

Fourth step: ASA (500 uM) left at room temperature for 2 days decomposes into Anhydride I and II

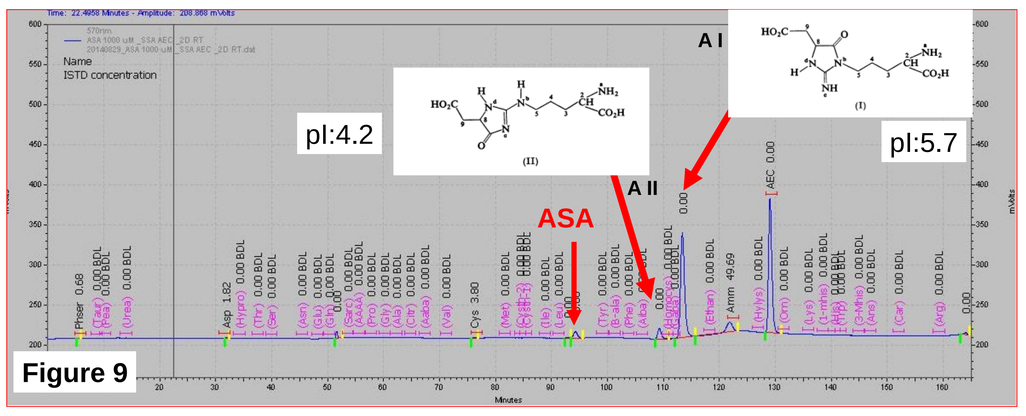


Figure 9

Figure 10: Plasma AA chromatogram with AEC – IS : the case diagnosed as ASA

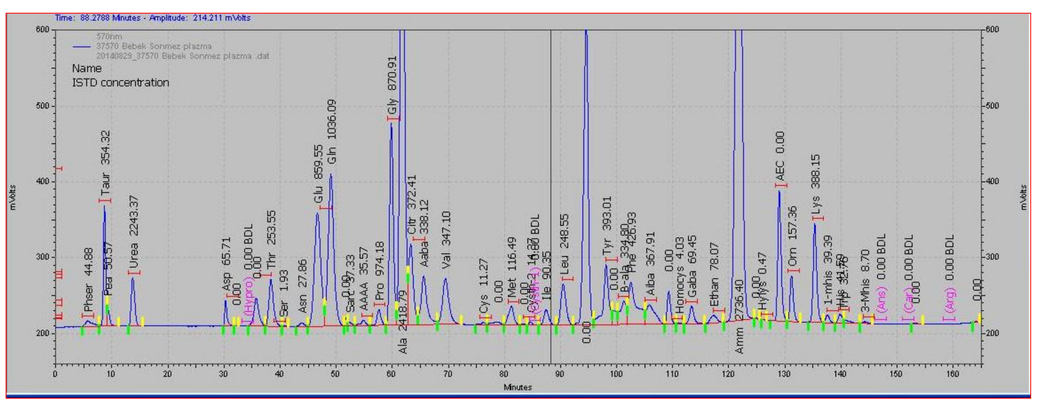


Figure 11: Urine AA chromatogram with AEC – IS : the case diagnosed as ASA

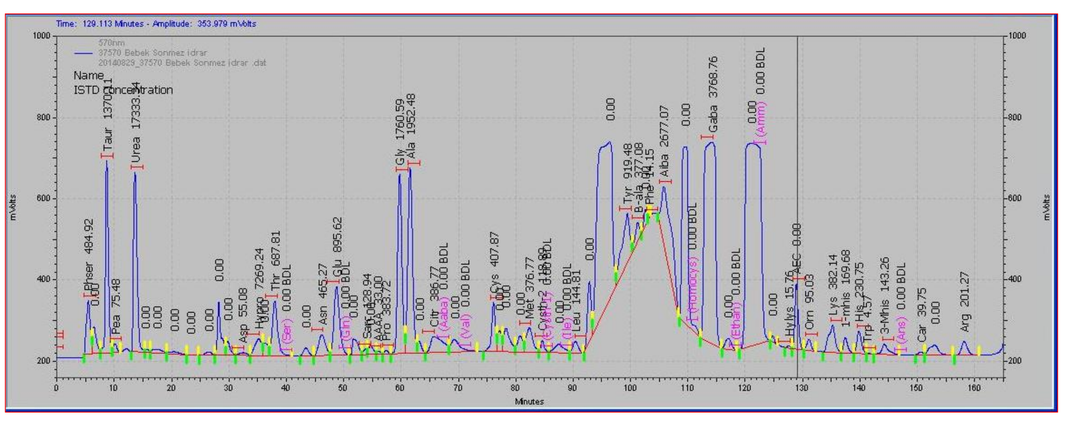


Figure 12: Plasma AA chromatogram with NLEU – IS of the same case diagnosed as ASA

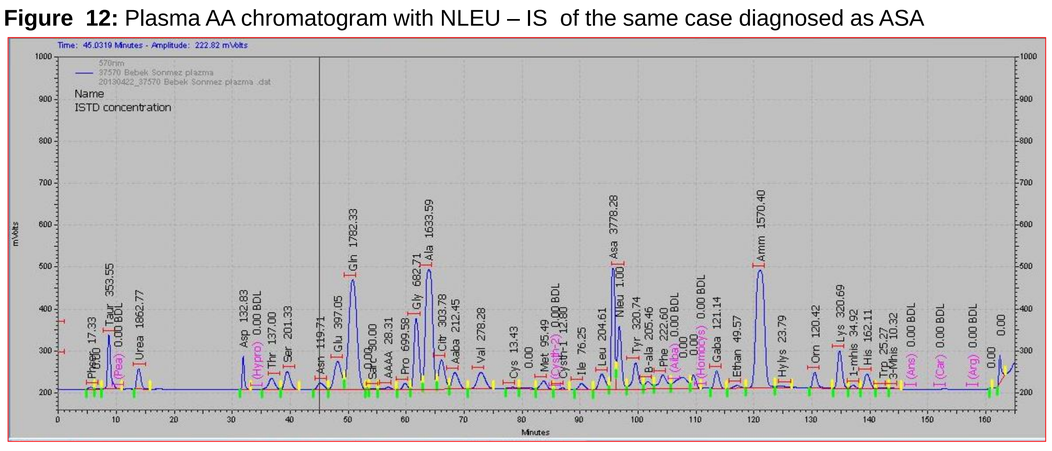
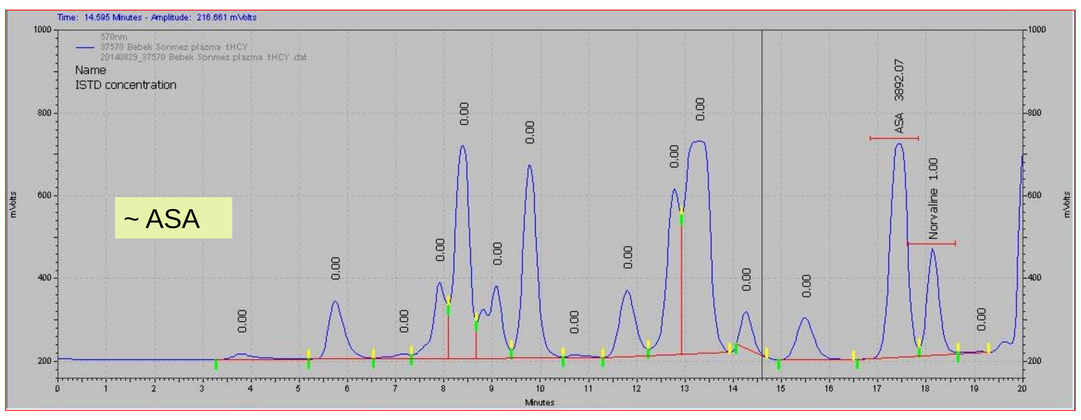
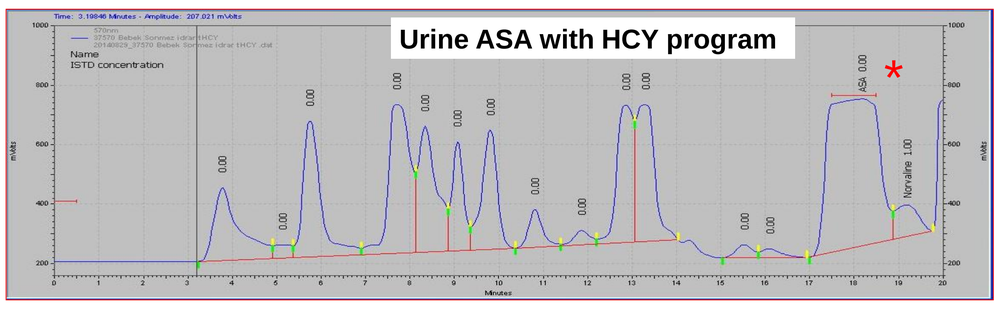


Figure 13: Plasma ASA quantification of the same case by using HCY short program



RESULT for ASA = ~ ASA (3892 uM in HCY program norvaline IS) – MET (-95 uM in full AA program with Nleu IS) or (-116 uM with AEC IS) = 3797 uM or 3776 uM



CONCLUSION

1-ASA (argininosuccinic acid) can be easily detected by full chromatogram as a sharp peak around 94 minutes
2-There is always a risk for NLEU (norleucine) users around ASA –Nleu retention time in the presence of drug treatment
3-Aminoethyl cysteine (AEC) as an internal standard is prefered since its elution is achieved at 128.9 minutes
3-For Nleu users ASA quantification can be obtained rapidly and accurately by using homocysteine (HCY) program
4-The value of plasma ASA obtained by HCY program can be easily corrected by substracting the plasma methionine value obtained by the standard AA program.
5-ASA quantification in urine needs dilution steps for urine * and anhydride I and II formation rate depends on medium pH and temperature