RAPID ANALYSIS OF CYSTINE FOR THE DIAGNOSIS OF RENAL CALCULI

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AIM

This study presents a rapid screening method for cystine measurement in urine with high peak resolution and accuracy and improved run time; one hour per sample .

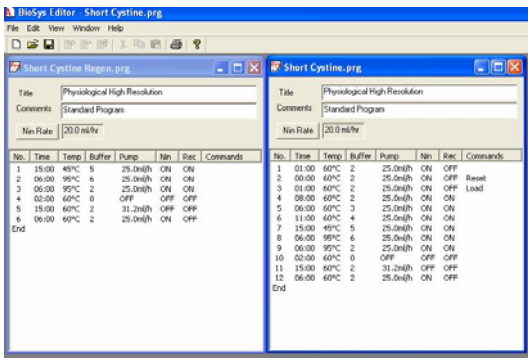
INTRODUCTION

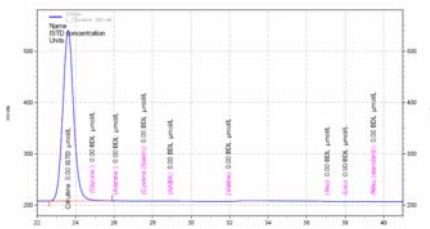
Classical cystinuria is the most common inborn error of aminoacid transport. The fact that the cystine is the least soluble natural aminoacid, its over excretion frequently results to the formation of cystine calculi in renal pelves, ureters and bladder along with infection, obstruction and with high risk of recurrent urinary stone formation that even may direct to renal failure. Cystine stones are difficult to treat and requires lifelong therapy. Diagnosis by quantification of cystine in urine and monitoring the patient with cystine excretion have a great impact on this disorder.

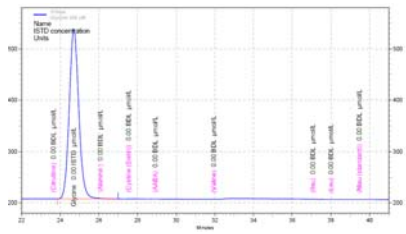
METHOD

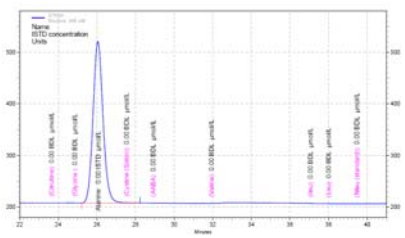
This short program we developed by using Biochrom 30 Aminoacid analyser enables 15 analysis to be performed a day. The separation is achieved using 20 X 4.6 mm physiological high resolution column using predominantly buffer CII (pH: 3.15). Norleucine is used as an internal standard. The program is shown below .
Single aminocid standards are run by the program to identify the peaks first , than master mix of a working standard as a mixture of amino acids , acidics and Neutrals A6407-sigma and Basics A6282 –sigma with SSA-Norleucine is prepared and run by the program .The single peaks of single amino acid standards (500 µM) prepared in loading buffer and the working standard containing aminoacids 500 µM in final concentration and cystine 250 µM are shown below .

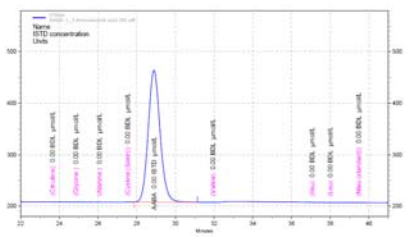
RESULTS

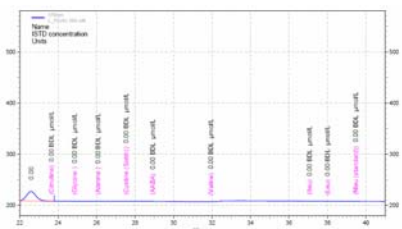




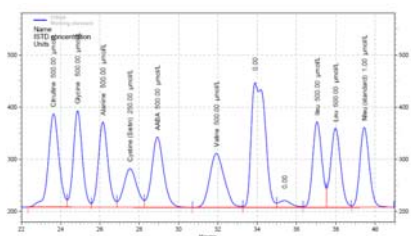


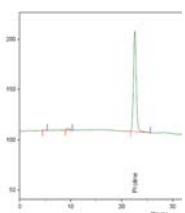






Proline 440 nm





440 nm
L_proline 500 uM

METHOD COMPARISON

11 real patient urine samples with a different wide range of cystine results are assayed by both full amino acids program and short cystine program . The data was checked for distrubution and it was non parametric.

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Urine_AA	,331	11	,001	,732	11	,001
Short_Cystine	,311	11	,004	,757	11	,003

a. Lilliefors Significance Correction

Ranks

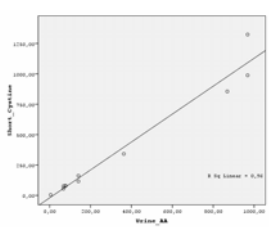
		N	Mean Rank	Sum of Ranks
Short_Cystine - Urine_AA	Negative Ranks	6[a]	5,50	33,00
	Positive Ranks	5[b]	6,60	33,00
	Ties	0[c]		
	Total	11		

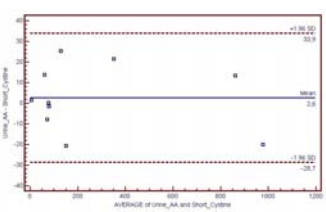
a. Short_Cystine < Urine_AA
b. Short_Cystine > Urine_AA
c. Short_Cystine = Urine_AA

Test Statistics[a]

	Short_Cystine - Urine_AA
Z	-,007[b]
Asymp. Sig. (2-tailed)	1,000

a. The sum of negative ranks equals the sum of positive ranks.
b. Wilcoxon Signed Ranks Test





The results of both programs showed a strong linear correlation as shown above by linear regression analysis

Bland_Altman Bias plot showed that all 11 points are between 1.96 SD

COMPARISON BETWEEN THE FULL AMINO ACID PROGRAM WITH SHORT CYSTINE PROGRAM.

The bias has been considered by means of the mean/median values obtained from participating labs in the ERNDIM Scheme 2010

PT testing material	ERNDIM QA 2010	Lab no 392		
		Cystine result in Full programme µmol/L	Scale Standard Deviations	Cystine results in Short Cystine Programme µmol/L
150	Number of labs enrolled = 169 Mean =42,7 Median=43,0 SD=5,19	45,2	0,0 - 0,5 SD	52,43
151	Number of labs enrolled = 175 Mean=66,8 Median=67,0 SD=8,15	63,32	(-0,5) - 0,0 SD	56,56
152	Number of labs enrolled = 173 Mean=31,7 Median=32,0 SD=3,78	29,48	(-1,0) - (-0,5) SD	26,16

CONCLUSION

This short program will ease the work load of clinical laboratories that have a continuos requirement for the analysis of urine specimens from patients with renal stones to screen for cystinuria or follow up the patients under treatment. Concerning the cost effectivity ,short cystine program will be a useful tool for effective time saving and efficient reagent usage.