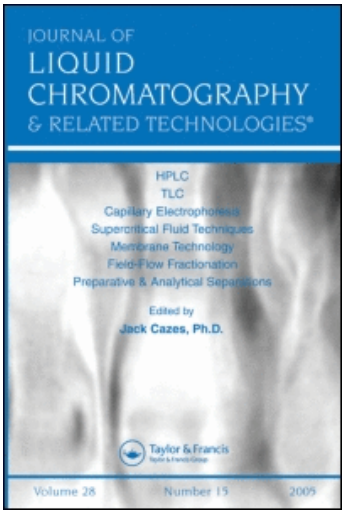

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Ibrahim Z. Atamna[a]; Gary M. Muschik[a]; Haleem J. Issaq[a]
[a] Program Resources, Inc. NCI-FCRF, Frederick, Maryland

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A DIRECT INJECTION PROCEDURE FOR THE DETERMINATION OF PROLINE IN UNEXTRACTED URINE WITH MICELLAR HYDRO-ORGANIC MOBILE PHASES CONTAINING COPPER IONS

IBRAHIM Z. ATAMNA*, GARY M. MUSCHIK
AND HALEEM J. ISSAQ
Program Resources, Inc.
NCI-FCRF
P.O. Box B
Frederick, Maryland 21701

ABSTRACT

The direct injection of untreated urine and subsequent analysis by HPLC of proline was possible by using a micellar mobile phase modified with 8% 1-propanol and a C_{18} reversed phase column. The mobile phase contained 0.03M SDS, 0.01M acetate buffer and 0.001M Cu (II) ions. In situ detection of proline at 235nm was facilitated by the formation of a proline-copper complex. The analysis of other amino acids by this method is demonstrated.

INTRODUCTION

The separation and quantification of amino acids (AAs) have been studied for many years by different analytical procedures. Traditionally, the determination of AAs was performed by amino acid analyzers which are based on ion exchange mechanisms followed by post-column derivatization. TLC (1), GC

*Author to whom correspondence should be addressed.

(2) and more recently CZE (3) were also used. HPLC methods require pre-column fluoresence derivatization for detection. The above techniques require sample cleanup or extraction before derivatization or injection.

Recently Grushka et al (4) described a new approach to the separation of free amino acids using HPLC. In this approach the analysis of AAs was accomplished by in-situ formation of their copper charge transfer complexes, which have maximum UV absorption at 235 nm with a molar absorptivity of approximately 6000.

For routine analysis of biological fluids, conventional HPLC methods have drawbacks such as lengthy analysis time and tedious sample preparation. They generally require extraction of the compound of interest from the complex matrix. These steps considerably increase the possibility of error due to either incomplete extraction or incomplete matrix (protein, lipid, etc.) precipitation which may lead to column clogging. To overcome these problems researchers (5-8) have used different micelles above their critical micelle concentration (CMC). Their powerful solubilizing properties allow direct injection of serum and urine onto the chromatographic column without column clogging or pressure build-up.

It would be advantageous to be able to apply concurrently the use of micelles and copper ions for the direct injection and UV detection of proline and other amino acids in urine specimens. This study deals with the development and application of such a procedure. Also, the effect of 1-propanol content in the mobile phase on the column efficiency was examined.

EXPERIMENTAL

Materials

The acetate buffer was freshly prepared in the laboratory using an analytical grade sodium acetate (Fisher Scientific Company, NJ) and glacial

acetic acid (J. T. Baker Chemical Company, NJ). The pH was monitored using a Fisher Scientific pH meter. Analytical grade cupric acetate was obtained from Fisher Scientific Company. All the amino acid standards were purchased from Sigma Chemical Company. The column used was an RP-18, 5 μ spherical 250 x 4.6 mm (Phenominex, CA).

Instrumentation

A Hewlett-Packard liquid chromatograph (Model 1090M) equipped with a photodiode array detector, an autosampler, a computing control system (Chem. Station) and an oven was used in this study.

RESULTS AND DISCUSSION

In our work, there was a need to develop a simple, fast and quantitative assay for proline in urine. Since approximately 2000 specimens will be analyzed, the procedure should preferably have minimum sample handling (extraction, cleanup, derivatization, etc.). Literature surveys showed that most of the procedures used are time consuming. However, Grushka et al (4) were able to detect, by UV, free amino acids by forming their copper complexes in-situ. Additionally, Cline Love et al (7) were able to directly inject serum and urine samples for drug monitoring by HPLC by using micellar mobile phases.

In this study, it was believed that the combination of micellar mobile phases and in-situ copper complexation of proline might lead to a fast and simple quantitative procedure which is aminable to the analysis of a large number of samples and may be automated.

The micelle selected was 0.03 M sodium dodecyl sulfate (SDS). A 0.01 M acetate buffer of pH 5.3-5.6 was used as the mobile phase modified with 1-propanol (8% v/v). The advantages of this mobile phase were described earlier (4,6).

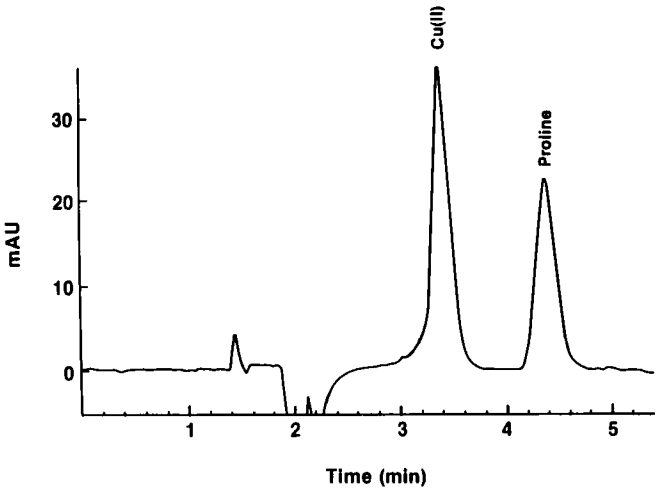

Figure 1 Chromatogram of standard proline (2 x 10^{-3} M). The 0.01 M acetate buffer (ph = 5.3) contained 10^{-3} M Cu (II) ions and 0.03 M sodium dodecyl sulfate (SDS) and 8% (v/v) 1-propanol. The temperature was 40°C, flow-rate = 1.5 ml/min.

Figure 1 is a chromatogram of a 10 µl injection of standard proline (2 x 10^{-3} M). The peaks at approximately 3.4 and 4.3 min. are those of copper and the proline complex, respectively. This shows that the procedure developed meets the required objective of a fast analysis time for proline. The next step was to see if this method applies to the analysis of proline in urine samples. Figure 2 shows a chromatogram of the urine sample. The peaks elutiny before 2.5 minutes are those of proteins in urine. Expansion of the peaks elutiny after 3.5 min. (Figure 3) shows the presence of several peaks.

The proline peak, which is separated from the other peaks, was identified by spiking the urine sample with proline standard. Quantification of proline was carried out by measuring the area under the peak. A calibration curve of proline standards is shown in figure 4. A linear relationship was observed in the range of 10-300 mg/liter. The minimum detection limit was 10 µg/ml. This detectable quantity, although satisfactory for our experimental needs, is not as sensitive as published procedures.

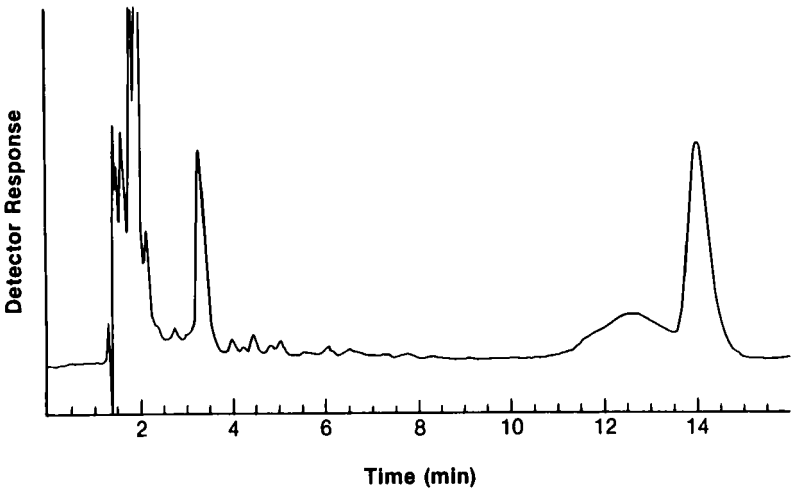

Figure 2 A chromatogram of 10 µl urine sample, chromatographic conditions as in Figure 1.

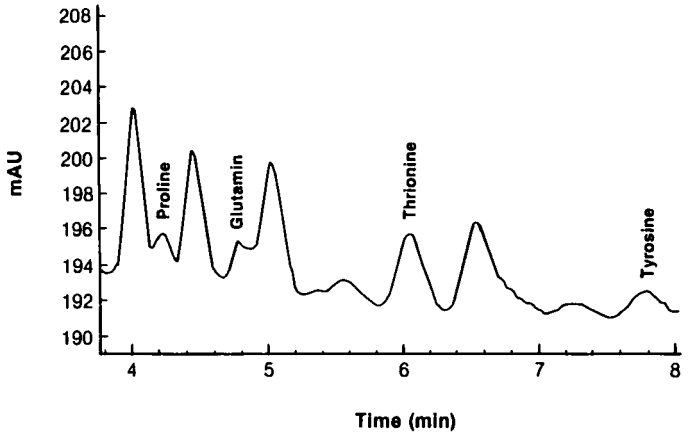

Figure 3 An expanded part of the chromatogram in Figure 2 to observe the amino acids of interest.

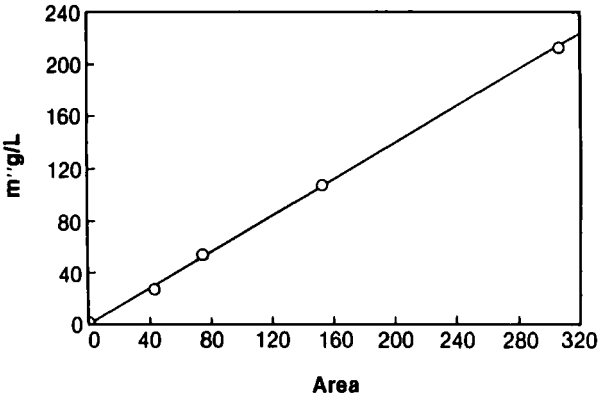

Figure 4 A calibration curve for standard proline, chromatographic conditions as in Figure 1. The calculated area is in arbitrary units.

Figure 5 shows a chromatogram of another urine specimen. It is clear that this procedure meets the objectives stated previously. Figures 3 and 5 showed the presence of peaks other than proline. It was of interest to determine if these peaks were amino acids present in urine. Figure 6 is a chromatogram of a standard mixture of AA that eluted within the time range of the peaks observed in Figure 5. While some amino acids eluted early, close to the dead volume; others eluted after 30 min. (see Table 1). Comparison of figures 5 and 6 indicate the presence of glutamine, thrionine and tyrosine in the urine sample. It is possible, that AAs other than proline can be quantified by this procedure.

Effect of Propanol Content on Column Efficiency

Recent studies (9,10) have demonstrated that the chromatographic efficiency of micelle containing systems could be increased to comparable efficiencies seen in traditional columns by adding small amounts of alcohol (MeOH, 1-PrOH) and operating at elevated temperatures (40°C). It is presumed that the stationary phase was not wetted in the absence of alcohol, resulting

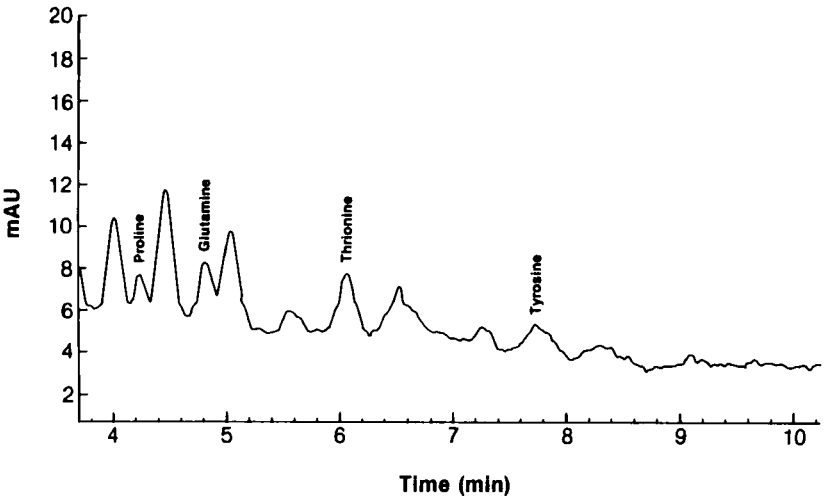

Figure 5 A chromatogram of another urine sample. Chromatographic conditions as in Figure 1.

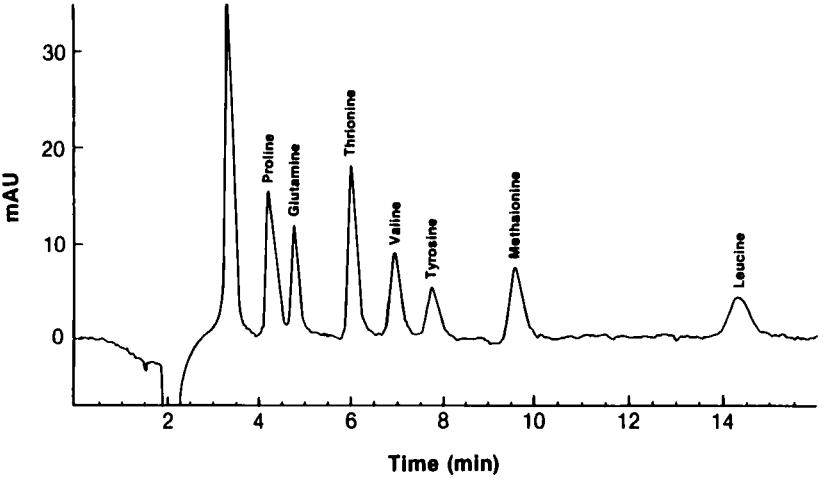

Figure 6 A chromatogram of amino acids standard mixture. Chromatographic conditions as in Figure 1.

TABLE 1

Elution times of amino acids using the present system

Amino acid	elution time (min.)
Aspartic acid	2.82
Glutamic acid	3.0
Alanine	3.2
Proline	4.3
Glutamine	4.8
Thrionine	6.0
Valine	6.9
Tyrosine	7.7
Methaionine	9.6
Leucine	14.3
Arginine	
Histidine	
Lysine	greater than
Cysteine	30 minutes
Tryptophan	
Phenylanine	

TABLE 2

Effect of % 1-propanol in the mobile phase on efficiency

% 1-propanol (v/v)	N(meter^{-1})
2	6980
4	6800
8	7560
10	8600

in slow mass transfer. In our study it was of interest to examine the effect of 1-propanol (2-10%) on column efficiency. It is important to mention here that 10% 1-propanol cannot be exceeded because of the possibility of precipitation of proteins from urine samples.

Table 2 shows the number of theoretical plates for the proline peaks at 40°C and different percentages of 1-propanol. Although there was an increase in efficiency with the increase of propanol in the mobile phase from 2% to 10%, this increase is not as large as expected with conventional HPLC columns. However, increasing the amount of 1-propanol leads to longer column life.

CONCLUSIONS

This method of direct injection of untreated urine and in-situ complexation of the AA with copper ions for direct UV detection is a promising procedure for selective AA quantification. The present study is useful for quantification of proline and other amino acids that are present in relatively large quantities (ppm) compared to other HPLC procedures where fluorescence is the mode of detection.

ACKNOWLEDGEMENTS

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REFERENCES

1. Issaq, H.J. and Devenyi, T., J. Liquid Chromatography, 4(12):2233, 1981.

2. Bayer, E., Gran, E., Kaltenegger, B. and Uhmann, R., Anal. Chem., 48, 1106, 1976.

3. Yu, M. and Dovichi, N.J., Anal. Chem., 61, 37-40, 1989.

4. Levin, S. and Grushka, E., Anal. Chem., 57, 1830-1835, 1985.

5. Aronyanart, M. and Cline Love, L.J., J. of Chromatogr., 342, 293-301, 1985.

6. Barford, R.A. and Sliwinski, B.J., Anal. Chem., 56, 1554-1556, 1984.

7. Cline Love, L.J., Zibas, S., Noroski, J. and Arunyanart, M., J. of Pharmaceutical & Biomedical Analysis, Vol. 3(6), 511-521, 1985.

8. Pramauro, E. and Pelizzetti, E., Trends in Analytical Chemistry, Vol. 7(7), 1988.

9. Dorsey, J.G., DeEchegaray, M.T. and Landy, J.S., Anal. Chem., 55, 924, 1983.

10. Henze, W.L. in "Ordered Media in Chemical Separations", ACS Symposium Series, 342, 1987, Edited by Willie L. Hinze and Daniel W. Armstrong, pp. 21-29.