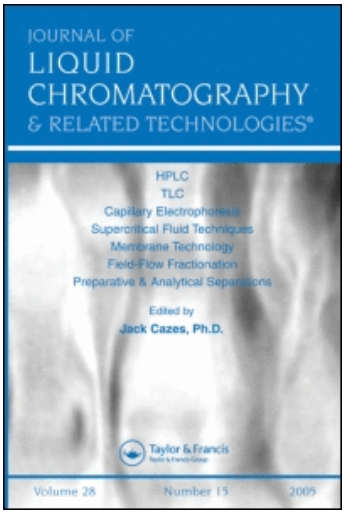

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

ANALYSIS OF 5-HYDROXYINDOLEACETIC ACID IN HUMAN FLUIDS BASED ON ANION EXCHANGE HPLC

Zak K. Shihabi[a]; Mark E. Hinsdale[a]
[a] Pathology Department, Wake Forest University Baptist Medical Center, Winston-Salem, NC, U.S.A.

Online publication date: 13 July 2000

To cite this Article Shihabi, Zak K. and Hinsdale, Mark E.(2000) 'ANALYSIS OF 5-HYDROXYINDOLEACETIC ACID IN HUMAN FLUIDS BASED ON ANION EXCHANGE HPLC', Journal of Liquid Chromatography & Related Technologies, 23: 12, 1903 — 1911
To link to this Article: DOI: 10.1081/JLC-100100461
URL: http://dx.doi.org/10.1081/JLC-100100461

J. LIQ. CHROM. & REL. TECHNOL., 23(12), 1903–1911 (2000)

ANALYSIS OF 5-HYDROXYINDOLEACETIC ACID IN HUMAN FLUIDS BASED ON ANION EXCHANGE HPLC

Zak K. Shihabi, Mark E. Hinsdale

Pathology Department
Wake Forest University Baptist Medical Center
Winston-Salem, NC 27157, USA

ABSTRACT

5-Hydroxyindoleacetic acid (5HIAA), a metabolite of serotonin is utilized clinically to detect the carcinoid tumors. A simple method for determination of 5HIAA in urine, serum, and cerebrospinal fluid (CSF) is described. For quantitation in urine, the method is based on a single step extraction followed by separation on an anion exchange short column. This type of column enabled the direct injection of the organic solvent on the column without any interference in the spectra or symmetry of the peak. For serum and (CSF) analysis acetonitrile deproteinization was used with injection on the same column. 5HIAA was detected by its native fluorescence which was more sensitive and more suited for daily routine analysis compared to the more common electrochemical detection. Reference range for 5HIAA in different human fluids is determined. Patients with carcinoid syndrome showed elevated levels of 5HIAA in both urine and serum.

INTRODUCTION

Serotonin (5-hydroxytryptamine) is a smooth muscle stimulant. In the brain it is a neurotransmitter, which is involved, in many behavioral patterns such as sleep, pain, and depression. It is deaminated to 5-Hydroxyindoleacetic acid (5HIAA) and excreted in the urine. The excretion of this 5HIAA increases

in patients with the carcinoid syndrome, a malignant tumor of the gastrointestinal tract. Serial determination of the urinary level of this compound is used to follow the progress of this tumor and the efficacy of the therapy. 5HIAA is also important in the study of the pharmacological action of drugs.

Several methods based on HPLC with either electrochemical detection[1-6] or fluorescence[6,7-9] have been described for the analysis of 5HIAA based on the separation on reversed-phase columns. The majority of these methods require complicated sample clean up such as double extraction or removal of the organic phase by evaporation. Organic solvents in the sample have to be removed before injection on the reversed-phase column because they have very strong absorption in the ultraviolet range, which masks the analyte spectra. In addition to this, they form a short gradient, especially on short columns, which results in non-symmetrical peaks with very poor reproducibility. As we illustrate here for the analysis of 5HIAA, solvent evaporation can be eliminated if an anion exchange column is used. Ion exchange columns are not common in routine analysis compared to the reversed-phase columns. A short anion exchange column in conjunction with fluorescence detection, allows for direct injection of small amounts of organic solvents without interferences from the organic solvent directly. Thus, the method can be greatly simplified by using a single step of extraction with the injection of the organic phase directly on the column while eliminating many of the common interferences.

The short anion exchange column is less expensive and offers rapid separation with a very low pressure drop compared to longer columns. Most of the methods for analysis of 5 HIAA utilize electrochemical detection; however, we show that the native fluorescence, especially with the new generation of detectors, has several advantages such as better baseline and better sensitivity with less interferences. We apply, here, the described method of analysis of 5HIAA in different human fluids such as urine, serum, and CSF.

EXPERIMENTAL

Reagents

Buffer: Formic acid, 6 g/L, pH 3.5. Extraction Solvent: Chloroform 200 mL, n-amyl alcohol 100 mL, ethyl acetate 100 mL were mixed and stored refrigerated. Pump solvent: Sodium acetate 7 g and NaCl 2 g were dissolved in 1000 mL water. The pH is adjusted to 5.7 with 2 mol/L HCl. Stock standard: 5-HIAA (Sigma Chemicals, St. Louis, MO) 100 mg/L with the addition of 3 drops of concentrated HCl.

Working standard: for urine 20 mg/L 5HIAA; and for serum/CSF 200 μg/L.

Apparatus

Pump: Model 110 A pump (Beckman Instruments, Palo Alto, CA) was used at a flow of 1.5 mL/min. Column: A cartridge column Aquapore Brownlee AX 300, 7 μm average particle size, 30X 4.6 mm (Perkin-Elmer, Norwalk, CT) was used for the separation Detector: A Model RFL-10AXL Fluorescence detector (Shimadzu Scientific Instruments, Columbia, MD) was set at excitation 280 nm and emission 330 nm.

Procedure

Urine

Add 25 μL urine, or standard to 500 μL formic acid buffer and 500 μL of extraction solvent. Vortex for 30 s and spin for 20 s. Inject 5 μL of the organic layer on the column.

Serum/Cerebrospinal Fluid (CSF)

Add 50 μL sample to 100 μL acetonitrile. Vortex-mix for 10 s and centrifuge at 13,000 X g for 20 s. Inject 20 μL on the column.

RESULTS AND DISCUSSION

Urine

5-Hydroyindole acetic acid is a weak water soluble acid. The distribution of 5HIAA between the organic extraction solvent and the formate buffer is about 1.12/1. The concentration of this compound can be increased in the organic solvent by keeping the added amount of the buffer to the minimum. However, because of the great intensity of the fluorescence of 5HIAA we elected to decrease the amount of 5HIAA transferred from the urine to the organic solvent by increasing the ratio of the buffer to the sample. Figure 1 illustrates a typical separation of a urine sample and a standard. The peaks are symmetrical and reproducible indicating that the injection of the organic solvent on this type of column did not affect the separation, the baseline, or the fluorescence intensity. The separation is complete in about 5 min. The CV for 6 samples at 5.4 mg/L is 3.9%. Spiking random urine samples with a standard of 10 mg/L gave a recovery of 97.2% (n=4). The linearity was tested between 2-20 mg/L. No interferences were encountered in the analysis of about 100 urine samples. The reference range for 39 random samples of urine from normal individuals as a ratio to creatinine excretion is 1.2–8.4 mg/g creatinine. This range is close to that reported previously.[2,5,8,13] Six samples from patients with carcinoid tumors all had elevated values of 5-HIAA above the reference inter-

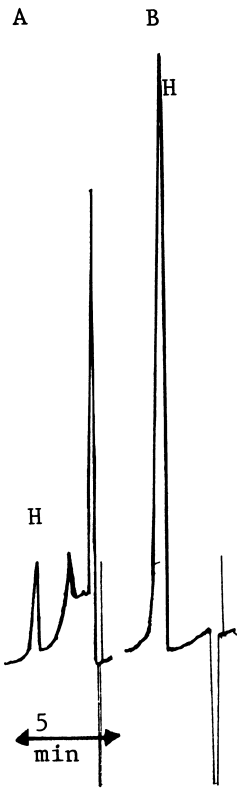

Figure 1. A chromatogram of: A) Urine from a normal individual, 3.3 mg/L 5 HIAA (H) and B) Standard, 20 mg/L.

Table 1

Comparison of 5HIAA* by HPLC with a Colorimetric Assay (13) for 6 Patients with Carcinoid Tumors

Patient	Color	HPLC
1	17	18
2	111	130
3	53	48
4	14	19
5	19	21
6	63	73
Reference range	0-6	1.2-8.4

* mg/g cretinine

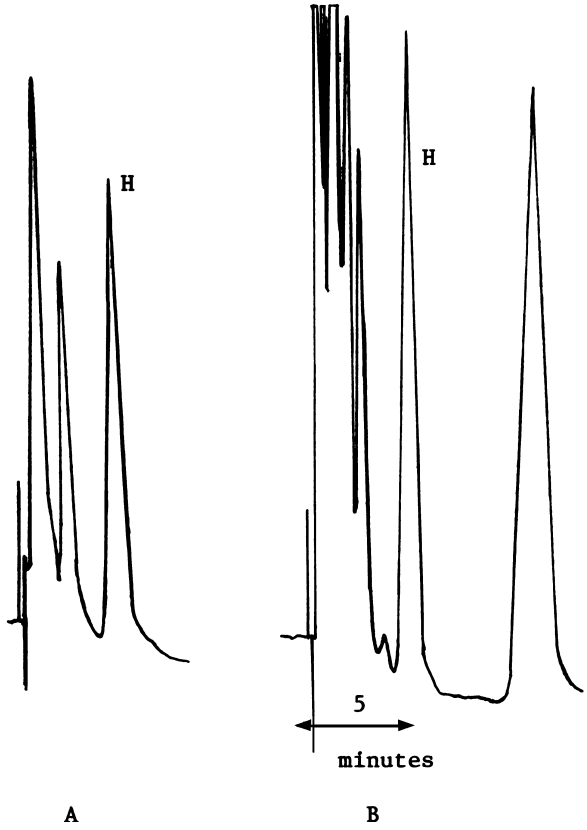

Figure 2. A chromatogram of a urine sample: A) extracted as described and B) non-extracted (diluted in the buffer), 5HIAA (H).

val. These values also compared well to a colorimetric method based on the nitrosonaphthol reaction,[13] Table 1.

Although many urine samples can be analyzed directly with simple dilution of the urine on this column few have many interfering or late eluting peaks necessitating sample extraction as illustrated in Figure 2. The single extraction step does not just decrease the interfering peaks but also indirectly speeds up the analysis time on the instrument by eliminating the late eluting peaks.

Serum

The concentration of 5HIAA in serum is about 200 times lower than that in the urine. Also, the serum contains a high concentration of proteins, which need removal before sample injection. Thus, the method has to be slightly modified for serum. Sample deproteinization with acetonitrile was found to be suitable for the assay. Figure 3a illustrates the separation of 5HIAA in serum. The recovery of 100 μg/L was 85%. The reference range for 29 serum samples is 0-43 μg/L. This range is close to 5HIAA values found in serum.[1,10] Three samples from patients with carcinoid syndrome have values of 210, 233, and 330

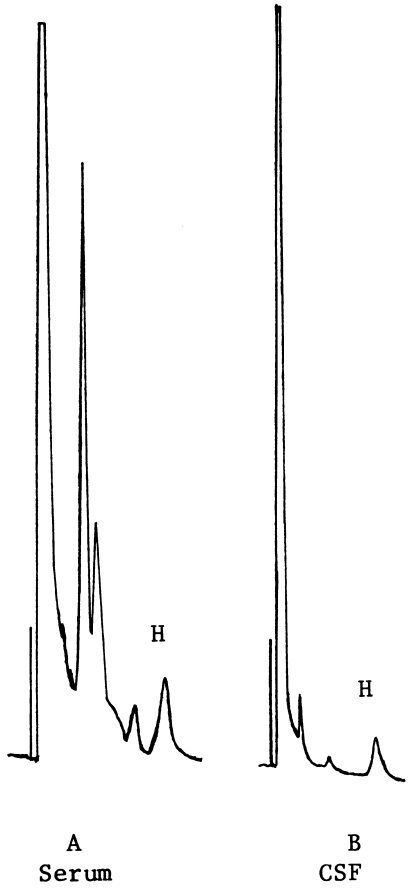

Figure 3. A chromatogram of: A) serum from a normal individual, 5 HIAA 43 μg/L, and B) CSF from a patient 23 g/L.

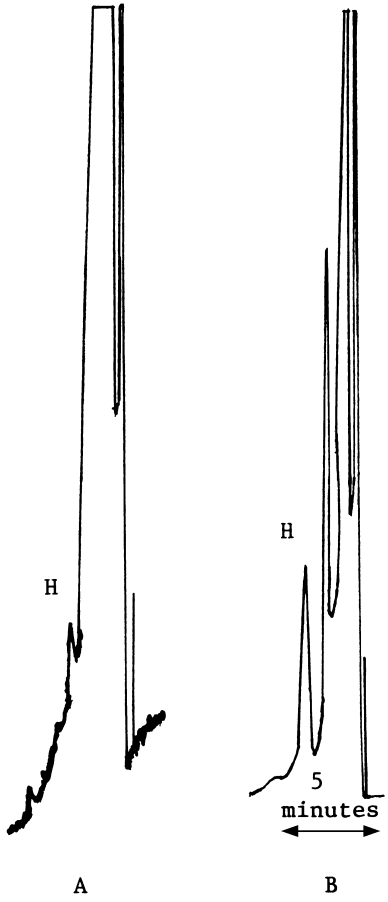

Figure 4. Comparison of the fluorescence detector to electrochemical (2) detection (at 0.95 V glassy carbon electrode). Both detectors were connected to the same column and the same pump: A) Electrochemical detection and B) Fluorescence detection; 5HIAA, H 8.3μg/L.

μg/L. Serum 5HIAA levels in the carcinoid syndrome have not been reported before; however, this data shows elevated values to be present in this disease. The serum level may be useful for patient diagnosis and management.

CSF

The concentration of 5HIAA in the CSF is also very low; similar to serum. Thus, the same method used for serum was used also for CSF. Figure 3b illus-

trates the separation of 5HIAA in CSF. The CSF yields much cleaner chromatograms when compared to urine or serum. The reference range based on the mean (52 μg/L) and 2SD for 30 samples was 13-130 μg/L which is close to that reported before.[3,11,12] The recovery was 104%.

Since the majority of the methods use the electrochemical detection,[1-6] we tried this method also with the electrochemical detector. The sensitivity is about 5 times better with the native fluorescence detection with a better baseline (especially with the new generation of fluorometers) and with cleaner chromatograms, Figure 4. Also, in practical use, the fluorescent detector requires much less maintenance and is more dependable for daily use compared to the electrochemical detector. Artigas et al.[6] using an older model found comparable sensitivity of the fluorescence and electrochemical detection. Bearcroft et al.[7] found the native fluorescence to give cleaner chromatograms compared to the electrochemical detection.

REFERENCES

1. C. A. Ribeiro, M. D. Cotrim, M. T. Morgadinho, M. I. Ramos, E. S. Santos, T. Macedo , Cephalagia, **10**, 213-219 (1990).

2. Z. K. Shihabi, J. Scaro, Clin. Chem., **26**, 907-909 (1980).

3. J. Wagner, P. Vitali, M. G. Palfreyman, M. Zraika, S. Hout, J. Neurochem., **38**, 1241-1254 (1982).

4. A. R. Bonfigli, G. Coppa, R. Testa, I. Testa, G. De Sio, Eur. J. Clin. Chem. Clin. Biochem., **35**, 57-61 (1997).

5. E. Pussard, N. Guigueno, O. Adam, J. F. Giudicelli, Clin. Chem., **42**:1086-1091 (1996).

6. F. Artigas, M. J. Sarrias, E. Martinez, E. Gelpi, Life Sci., **37**, 441-447, (1985).

7. C. P. Bearcroft, M. J. Farthing, D. Perrett, Biomed. Chromatogr., **9**, 23-27 (1995).

8. T. J. Panholzer, J. Beyer, K. Lichtwald, Clin. Chem., **45**,262-268 (1999).

9. M. Kai, H. Iida, H. Nohta, M. K. Lee, K. Ohta, J. Chromatogr. B, **720**, 25-31 (1998).

10. M. J. Schwarz, M. Spath, H. Muller-Bardorff, D. E. Pongratz, B. Bondy, M. Ackenheil, Neuroscience Lett., **259**, 196-198 (1999).

11. S. Sjoberg, M. Eriksson, C. Nordin, Eur J. of End., **139**, 493-497 (1998).

12. L. K. Jacobsen, J. A. Frazier, A. K. Malhotra, F. Karoum, K. McKenna, C. T. Gordon, S. D. Hamburge, M.C. Lenane, D. Pickar, W. Z. Potter, J. L. Rapoport, Am. J. Psychiat., **154**,69-74 (1997).

13. Z. K. Shihabi, E. L. Wilson, Clin. Biochem., **15**,106-108 (1982).

Received November 28, 1999 Author's Revisions February 29, 2000
Accepted December 30, 1999 Manuscript 5200