*Journal of Chromatography*, 571 (1991) 231–234
*Biomedical Applications*
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6028

## Short Communication

# Rapid determination of 5-hydroxytryptamine in whole blood by liquid chromatography with fluorimetric detection

E. TRAIFFORT, P. HUBERT and N. TAYEB

*Unité de Pharmacologie Clinique, Centre Hospitalier Sainte-Anne, 1 Rue Cabanis, 75674 Paris Cedex (France)*

and

N. AYMARD*

*Unité de Pharmacologie Clinique, Centre Hospitalier Sainte-Anne, 1 Rue Cabanis, 75674 Paris Cedex (France)* and Laboratoire de Pharmacologie, Faculté de Pharmacie, Université René Descartes, 4 Avenue de l'Observatoire, 75270 Paris (France)*

(First received April 8th, 1991; revised manuscript received May 30th, 1991)

## ABSTRACT

A rapid and simple reversed-phase (using μBondapak $C_{18}$ as the stationary phase) liquid chromatographic method with fluorimetric detection is described for the quantitation of 5-hydroxytryptamine in whole blood. The rapidity and simplicity of the method are explained by the absence of a pretreatment. 5-Fluoro-*dl*-tryptophan was used as internal standard. The mobile phase was 0.01 *M* phosphate buffer (pH 4.5) with 0.0025 *M* 1-heptanesulfonic acid and 20% methanol. The detection wavelengths were 302 nm for excitation and 340 nm for emission. Analysis time was 10 min with retention times for 5-hydroxytryptamine of 9 min and for 5-fluoro-*dl*-tryptophan of 7 min. This method is proposed for biological exploration of psychiatric disorders involving 5-hydroxytryptamine and would be useful for tryptophan.

## INTRODUCTION

5-Hydroxytryptamine (serotonin, 5-HT), is a well known neurotransmitter implicated in certain mental disorders. Accessible forms of 5-HT in human are, in blood, those bound to platelets, where uptake and storage mechanisms have been shown to mimic those described in the central nervous system [1,2]. Analytical techniques described for measuring serotonin are currently high-performance liquid chromatographic (HPLC) systems with electrochemical [3–5] or fluorimetric [6] detection, radioenzymatic procedures [7] and radioimmunoassays [8].

We propose here a chromatographic analysis with fluorimetric detection that is easier to perform than the radioassays. This method allows a rapid, selective and sensible determination of 5-HT in whole blood, using 5-fluoro-*dl*-tryptophan (5-FdlT) as internal standard and secondary standards for the calibration curve.

0378-4347/91/$03.50 

These characteristics lead us to propose this method for the biological exploration of psychiatric disorders. This method would be useful for tryptophan assay in future studies.

## EXPERIMENTAL

### *Reagents and chemicals*

The reference compounds were: serotonin (5-hydroxytryptamine creatinine sulfate), *l*-ascorbic acid and perchloric acid from Fluka (Buchs, Switzerland); 5-FdlT from Sigma (St. Louis, MO, USA); Pic B7 (1-heptanesulfonic acid) from Waters Assoc. (Milford, MA, USA); potassium dihydrogenphosphate from Merck (Darmstadt, Germany); and methanol from FSA Laboratory (Loughborough, UK).

### *Apparatus*

Liquid chromatography was carried out using a pump (LC-6A Shimadzu), a 150 mm × 4.6 mm I.D. column packed with μBondapak $C_{18}$ particle size 5 μm (Waters) and a fluorimetric detector (Shimadzu RF-530).

### *Assay procedure*

Heparinized whole blood samples were immediately used for the assay or stored at −80°C. All experiments described above were performed on ice. A 450-μl aliquot of whole blood was mixed with 50 μl of a 10 ng/ml 5-FdlT solution, used as internal standard, and 100 μl of 1 *M* ascorbic acid, in order to prevent oxidation. The mixture was stirred on a vortex mixer for 10 s and then deproteinized with perchloric acid (100 μl of a 4 *M* solution). After vortex-shaking for 30 s, the mixture was centrifuged at 13 000 *g* for 10 min at 0°C. The resulting supernatant was filtered through a 0.22-μm filter, before injection into the chromatographic column.

A standard curve was obtained with pooled whole blood from healthy patients. Calibration points (secondary standards) were obtained in a manner similar to that previously described; the pooled whole blood was supplemented with increasing concentrations of 5-HT in order to obtained the following values: 0, 25, 50, 200, 400, 600 ng/ml. A standard curve was also obtained using primary standards as described by Anderson *et al.* [6].

The column was equilibrated with a mobile phase composed of 20% methanol, 0.01 *M* phosphate buffer and 0.0025 *M* Pic B7 reagent in deionized water. The pH was adjusted to 4.50 with 6 *M* sodium hydroxide. The flow-rate was 1.0 ml/min. The wavelengths were 302 nm for excitation and 340 nm for emission.

## RESULTS AND DISCUSSION

Fig. 1 shows a typical chromatogram of a whole blood sample. The 5-HT peak is perfectly resolved from Trp and from the internal standard, 5-FdlT. Total

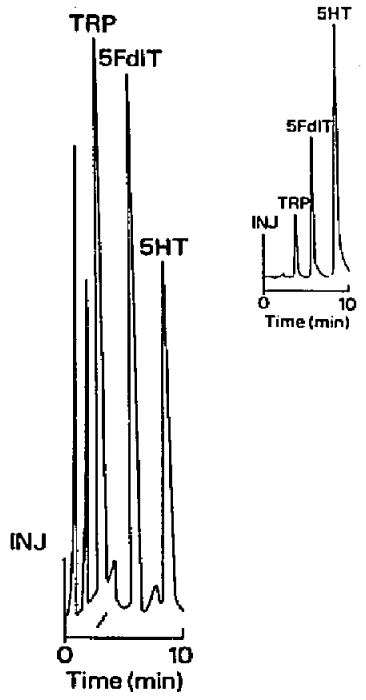


Fig. 1. Chromatogram of Trp, 5-FdIT and 5-HT in 450 $\mu$l of human whole blood. Inset: chromatogram of the reference compounds (primary standards).

analysis time was 10 min. Retention times for 5-HT, Trp and 5-FdIT were 9, 5 and 7 min, respectively. The 5-HT and Trp peaks were identified by comparing their retention times with those of primary standards.

A linear relationship between peak-height ratios (5-HT to internal standards) and concentrations of 5-HT (ng/ml) was observed between 25 and 600 ng/ml with a correlation coefficient, $r$, of 0.9997.

The detection limit for 5-HT was estimated to be 25 ng/ml for secondary standards and 1 ng/ml for primary standards. The day-to-day reproducibility obtained from ten determinations for a 5-HT concentration corresponding to 100 ng/ml led to a coefficient of variation (C.V.) of 7.0%. The within-day reproducibility obtained from twenty determinations for two 5-HT concentrations (50 and 500 ng/ml) gave a C.V. of 8.0%.

The assay described enables a rapid and simple determination of 5-HT in whole blood. The rapidity and simplicity of the method are explained by (i) the absence of a complex sample pretreatment using either alumina absorption [9] or anion-exchange resin [10] and the absence of an HPLC system with a column-switching technique [11]; (ii) the use of a mobile phase containing a counter-ion (Pic B7), which led to the elution of 5-HT at the end of the chromatogram, whereas other amines in blood are simultaneously eluted at the beginning of the chromatogram [12]; and (iii) the use of an organic modifier, methanol, which allowed the retention time of 5-HT to be reduced, since indolic compounds are

relatively non-polar and their retention time decreases as the concentration of methanol increases without modifying the elution order. Despite the lower sensitivity obtained with secondary standards (compared with primary standards), these are preferred because secondary standards and specimens are prepared under the same conditions.

The resolving power of HPLC is combined with the sensitivity and specificity of fluorimetry, which was used because of the natural fluorescence of 5-HT, Trp and 5-FdlT and because fluorescence detection requires less technical maintenance than electrochemical detection. 5-FdlT is an excellent internal standard, not existing in blood. The concentrations of serotonin in blood of fourteen healthy subjects were 242 ± 57 ng/ml (range 50–330 ng/ml), which is in accordance with the values obtained by other authors [6].

The specificity of this assay is also based upon the chromatographic separation technique. The identification of the peaks was achieved by addition of a small quantity of standard to a blood sample, which did not modify the characteristics of the peaks under assay.

The assay on whole blood presents various advantages: (i) the simplicity of sampling for clinical departments; (ii) the simplicity of sample pretreatment (the preparation of plasma with high and consistent yields of platelets is a difficult step); and (iii) the assurance that the entire platelet population is assayed since all of the 5-HT in blood is bound to platelets.

In conclusion, this method is suitable for the analysis of serotonin and eventually of tryptophan in biological samples, which are often required for the study of certain psychiatric disorders.

REFERENCES

1 J. M. Sneddon, *Prog. Neurobiol.*, 1 (1973) 151.
2 S. M. Stahl and H. Y. Meltzer, *J. Pharmacol. Exp. Ther.*, 205 (1978) 118.
3 N. M. Munoz, C. Tutins and A. R. Leff, *J. Chromatogr.*, 493 (1989) 157.
4 E. R. Korpi, *Clin. Chem.*, 30 (1984) 487.
5 K. Shibata and M. Onodera, *J. Chromatogr.*, 430 (1988) 381.
6 G. M. Anderson, J. G. Young, D. J. Cohen, K. R. Schlicht and N. Patel, *Clin. Chem.*, 27 (1981) 775.
7 I. Hammel, Y. Naot, E. Ben-David and H. Ginsburg, *Anal. Biochem.*, 90 (1978) 840.
8 M. R. Geffard, J. J. Prizillout and M. A. Delaage, *J. Neurochem.*, 39 (1982) 1271.
9 G. P. Jackman, V. J. Carson, A. Bobik and H. Skews, *J. Chromatogr.*, 182 (1980) 277.
10 P. Frattini, M. L. Cucchi, G. Sautagonisto and G. L. Corona, *Clin. Chim. Acta*, 92 (1979) 353.
11 I. Morita, *J. Chromatogr.*, 526 (1990) 367.
12 J. H. Trouvin, A. Gardier, G. El. Gemayel and C. Jacquot, *J. Liq. Chromatogr.*, 10 (1987) 121.