Liquid-Chromatographic Determination of Serotonin in Serum and Plasma

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Using high-performance liquid chromatography with electrochemical detection, we determined serotonin in plasma from parkinsonian patients being treated with L-3,4-dihydroxyphenylalanine or N-[(2,3,4-trihydroxybenzyl)hydrazine hydrochloride plus L-3,4-dihydroxyphenylalanine ("Sinemet") and in serum from a blood bank, from "normal" persons, and a pooled specimen from a hospital clinical laboratory. The values obtained for the two groups of Parkinson's disease patients showed no significant difference. Long-term storage on solid CO2 was shown to be an adequate technique for preserving samples. The mean (±SEM) normal value obtained for serotonin in serum was 146 ± 46 μg/liter (n = 23), a result in harmony with that previously obtained [Clin. Chem. 20, 812 (1974)] by fluorometry. In comparison to other methods for measurement of serotonin in serum or plasma, we believe that the present scheme offers greater selectivity, sensitivity, and precision.

Additional Keyphrases: normal values • variation, source of • nervous-system disease • parkinsonism

Serotonin, a well-known neurochemical transmitter, has been strongly implicated in number of mental and physical disorders. There are many hypotheses concerning abnormal metabolism and function of serotonin as a causative agent for certain mental disorders (1). This has led to a great deal of research on animal models in which serotonin and related species are determined in brain tissue samples. Human studies, on the other hand, have focused upon more readily accessible forms of serotonin, e.g., that bound to platelets in the blood. Although they do not directly provide information about this substance in the central nervous system, such samples are certainly much more easily obtained than tissue or cerebrospinal fluid samples, and blood serotonin samples yield considerably more timely information concerning in vivo activities than, for example, urine. Moreover, the uptake and storage of serotonin in blood platelets has been shown to strongly mimic these same processes in central nervous system tissues.

Blood serotonin determinations have already been applied to studies of non-carcinoid malignancy (2), carcinoid syndrome (3, 4), Down's syndrome (5), Hermansky-Pudlak syndrome (6, 7), postcibal concentrations involving a possible hormonal role in gastrointestinal physiology (8), and to various blood disorders (9).

We therefore decided to try to apply our recently reported method (10) for the determination of serotonin in nervous tissue samples to a variety of blood samples. It is more sensitive and selective than are the more commonly used methods. We demonstrate the use of high-performance liquid chromatography with electrochemical detection for (a) a comparison of serotonin concentrations in the platelet-rich plasma of two groups of parkinsonian patients on different therapies, (b) an analysis of serum serotonin in a group of normals, (c) an analysis of serum serotonin in blood-bank and pooled serum samples, and (d) a study of the effect of storage on solid CO2 on serum serotonin values.

Materials and Methods

Apparatus

Homogenizer. All samples were routinely ultrasonicated, to disrupt any intact platelets, with a Model 200P ultrasonic homogenizer (Heat Systems-Ultrasonics, Plainview, N.Y. 11803), equipped with a long probe tip for small-volume work. This system was normally operated on a 50% duty cycle (alternately on and off for 1-s intervals, to minimize heating) for a total disruption time of about 30 s.

Shaking. Shaking was done at 280 oscillations per minute and 15 °C. Vials were always placed on their side with the long axis parallel to the direction of shaking.

Liquid chromatography. The high-performance liquid chromatography with electrochemical detection has been previously described in detail (10–12). The columns, detector, and other materials are now commercially available from a single source (Bioanalytical Systems, Inc., West Lafayette, Ind. 47906). Notable differences between this arrangement and other such devices are (a) the column, packed with DuPont Zipax SCX strong cation-exchange resin (DuPont Instruments, Wilmington, Del. 19898), was 3 mm (i.d.) × 750 mm, (b) the eluting solvent was a pH 5.1 citrate/acetate buffer, (c) the flow rate was about 1.3 ml/min, and (d) the working electrode potential was maintained at +0.60 V vs. the saturated calomel electrode.

Centrifuges. Platelet-rich plasma was prepared by centrifuging with a table-model Sorvall GLC-1 (DuPont Instruments Sorvall Operations, Newton, Conn. 06470). The aqueous and butanol layers in the solvent extraction (vide infra) were more cleanly separated with a Sorvall RC2B high-speed centrifuge operated at 7900 × g, obtained from the same manufacturer.

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Reagents

*n*-Butanol. Reagent-grade butanol from Mallinckrodt, Inc., St. Louis, Mo. 63147, was routinely utilized throughout this study. When obtained in supposedly comparable purity from other manufacturers, *n*-butanol has occasionally given very large solvent fronts in the liquid chromatography. Thus, all new batches of *n*-butanol should be tested before use. If a suitable batch cannot be found, the purification procedure described by Shore and Olin (13) is effective.

*n*-Heptane. “Baker Analyzed” heptane (J. T. Baker Chemical Co., Phillipsburg, N.J. 08865) was used for all solvent extractions. As with the *n*-butanol, this substance can be purified if necessary (13). However, we have encountered no problems with reagent-grade material from various manufacturers.

Citrate/acetate buffer. This buffer was used as the mobile phase in liquid chromatography and as a diluting agent for standards before solvent extraction. It is prepared by dissolving 8.2 g of anhydrous sodium acetate, 2.1 ml of glacial acetic acid, 4.8 g of sodium hydroxide, and 10.5 g of citric acid monohydrate (all from Mallinckrodt, Inc.) in 1 liter of water. This solution was filtered through a 0.45-μm Millipore (Bedford, Mass. 01730) filter before use, to prolong pump life.

Serotonin. Serotonin (5-hydroxytryptamine) creatinine sulfate monohydrate was obtained in the highest possible purity, from Regis Chemical Co., Morton Grove, Ill. 60053. All concentrations of serotonin are reported as the free base.

3,4-Dihydroxybenzylamine. The internal standard, 3,4-dihydroxybenzylamine hydrobromide, 98% pure, was obtained from Aldrich Chemical Co., Milwaukee, Wis. 53233.

Dilute HCl solution, 10 mmol/liter. Dilute 0.86 ml of AR grade concentrated HCl (Mallinckrodt, Inc.) to 1 liter with de-ionized water.

Stock standard solution. A stock standard containing about 10 mg of serotonin per liter is prepared by dissolving an appropriate amount of the salt, accurately weighed, in 100 ml of the dilute HCl solution. The HCl solution is previously de-aerated with oxygen-free nitrogen for about 15 min. The resulting mixture is typically stable for as long as one month when stored in the refrigerator at 4 °C. This solution is unstable when frozen and thawed.

Working standard solution. Just before an analysis, a 1.00-ml aliquot of the stock standard is diluted 100-fold with the dilute HCl solution, which has been previously de-aerated. Thus, this standard contains about 100 μg/liter, a concentration comparable to that in blood.

Internal standard solution. A solution containing 2.5 × 10^-6 mol of the internal standard (3,4-dihydroxybenzylamine) per liter is prepared by dissolving the appropriate amount of its salt in the dilute, previously de-aerated HCl. Solutions of this type are adequately stable for as long as one month when stored refrigerated at 4 °C. Knowledge of the exact concentration of the internal standard is not required, but the same volume of the same mixture must be used throughout individual determinations (all standards and samples in a single run) for maximal precision and accuracy.

Ascorbic acid solution. As in our previous (10) investigation of brain tissue, we added ascorbic acid to the working standards during analysis, as an antioxidant. This solution is prepared immediately before use by dissolving 11 mg of ascorbic acid in 1.0 ml of the dilute, de-aerated HCl.

Ethylenediaminetetraacetate (EDTA). A solution containing 100 mmol of EDTA per liter is prepared by dissolving 3.72 g of the anhydrous disodium salt and 1.0 g of NaOH in about 90 ml of water. Concentrated HCl, 2.15 ml, is then added and the mixture diluted to 100 ml with water.

HCl for homogenization. An HCl solution (25 mmol/liter) is prepared by diluting 2.15 ml of concentrated HCl (Mallinckrodt, Inc.) to 1 liter with deionized water. This solution was used only in the initial treatment (before homogenization) of blood samples.

Other compounds. All other compounds used in this investigation were obtained from either Aldrich Chemical Co. or Regis Chemical Co. in the highest purity available.

Water. All water employed was de-ionized.

Sample Sources and Collection

General. All samples from the below-mentioned groups (persons of both sexes) were taken only during normal waking hours, but at no specific time of day.

Parkinsonian patients. Persons in this category were divided into two separate groups: (a) those receiving L-3,4-dihydroxyphenylalanine only, consisting of 10 individuals with an average age of 65.8 years (range 55–80), and (b) those receiving “Sinemet,” consisting of 23 persons with an average age of 64.2 years (range 54–68). “Sinemet” is a combination of a peripheral decarboxylase inhibitor [N-(DL-seryl)-N'-(2,3,4-trihydroxybenzyl)-hydrazine, Hoffmann-LaRoche, Nutley, N.J., 01234] and L-3,4-dihydroxyphenylalanine.

Normal persons. This group, 23 persons of average age about 30–35 years, were all having blood drawn for the purpose of routine pre-employment physical examinations.

Pooled sera. This pooled sample was obtained from the hospital clinical laboratory.

Blood bank. Serum samples were obtained from a blood bank after having been stored at −10 °C for one to six months.

No patient history was available for any of the blood bank, the normal, or the pooled sera samples.

Sample collection. All samples collected by us were obtained by venipuncture with a new needle and plastic evacuated blood-collection tubes. The plasma samples (both groups of parkinsonian patients) were drawn in tubes containing EDTA to prevent clotting. After collection, these tubes were gently inverted a few times to assure mixing and promptly centrifuged at low speed, according to Crawford (3), to obtain the platelet-rich plasma. The platelet-rich plasma, after careful separation from the discarded cells, was stored on solid CO₂ until analysis (one to two weeks thereafter).

Serum samples from the normal individuals were allowed to clot for 15–30 min, then centrifuged at low-speed. The serum was then aspirated and frozen on solid CO₂ before analysis (within two weeks). The serum samples used for the pooled sera represent the combined remainder of such material obtained in a single working day from a routine hospital laboratory. At the end of the day, the pool was gently mixed and stored on solid CO₂ for analysis some one to three weeks later. Blood bank serum samples, simply used as received, were also stored frozen on solid CO₂ until analysis one to two weeks later.

Procedure

The samples (plasma or serum) are thawed, and 500 μl is transferred to a 30-ml screw-cap vial with an accurate pipet. The vial should already contain 100 μl of the 25 mmol/liter HCl solution prepared for use in homogenization. The mixture is subjected to ultrasonic treatment for 30 s and saturated with salt (1 g of NaCl/vial). A 12.0-ml portion of butanol is added, and the samples are shaken for 60 min at 280 oscillations per second and 15 °C. A 10.0-ml aliquot of the butanol (top) layer, obtained after allowing 10 min for the layers to separate (or 10 min of centrifugation at 7900 × g and 4 °C if better solvent front separation in the chromatography is desired), is transferred to a second 30-ml screw-cap vial containing 17.0 ml of heptane and 200 μl of the dilute (10 mmol/liter) HCl. The
second vial is shaken and 10 min later the non-aqueous (top) layer is discarded; a 50-μl portion of the aqueous layer is then chromatographed.

Working standards are treated the same as samples. The only exceptions are: (a) the initial solution, before ultrasonic treatment, utilizes 750 μl of the citrate/acetae buffer instead of the HCl; (b) 10 μl of the ascorbic acid solution is added to the initial mixture, to help prevent oxidation of the standards; and (c) shaking time for the initial extraction into butanol is shortened to 20 min, as previously shown to be applicable with standards (10).

Calculations and Statistics

All calculations are based on the peak heights of the resulting chromatographic components.

Ratios of the peak heights for serotonin and the internal standard are compared for samples and standards, as described before (10, 11). In accordance with prior studies, the final value for serotonin is obtained by multiplying the result of this calculation by 1.29, which corrects for the lower percentage recovery of this species in the presence of biological materials as compared to aqueous samples (10, 14).

For example, if a 500-μl aliquot of an unknown serum sample yielded a serotonin peak height of 30.0 mm and a 3,4-dihydroxybenzylamine peak height of 20.0 mm, the average working standard, after similar extraction, yielded corresponding values of 7.0 and 14.0 mm, and the standard contained 19.5 ng of serotonin (in the utilized 500 μl or 39 ng/ml), the calculation would be as follows:

Serotonin, μg/liter = [(serotonin peak/3,4-dihydroxybenzylamine peak)\text{vol. sample, ml} × (ng serotonin in std) × 1.29]/[(serotonin peak/3,4-dihydroxybenzylamine peak)\text{vol. sample, ml}]

\[
= \frac{(30.0)}{20.0} × 19.5 × 1.29
= \frac{(7.0)}{14.0} × 0.500
= 151 \text{ μg/liter}
\]

All uncertainties are expressed as the standard error of the mean (SEM). Statistical significance was examined by use of Student's \textit{t}-test.

Results

Serum Samples

Many previous studies have commented on proper storage for blood-serotonin samples. We examined the long-term stability of this substance in the largest sample we had, the pooled sera, by the simplest method: storage on solid CO₂. A typical chromatogram for such a sample is presented in Figure 1. Comparable chromatograms were obtained for the other samples (plasma and serum) used in this investigation. As a result of eight separate determinations on a total of 54 samples, we arrived at an average value of 98 ± 1 μg/liter for serotonin in the pooled serum samples. The consistency of the technique as well as the long-term stability on such storage is depicted in Figure 2.

We analyzed 23 samples from the normal individuals and found a mean value of 144 ± 46 μg of serotonin per liter (n = 26). The final group of serum samples, those from the blood bank, gave a mean of 102 ± 8 μg/liter.

Plasma Samples

Serotonin in platelet-rich plasma was determined for both the parkinsonian patients on L-3,4-dihydroxyphenylalanine therapy and those on “Sinemet” therapy. Table 1 presents the results for both groups.

Interferences

Due to its high selectivity, the high-performance liquid chromatograph with electrochemical detection is capable of providing the investigator with assurance that potential in-

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**Table 1. Platelet-Rich Plasma Serotonin in Parkinsonian Patients**

<table>
<thead>
<tr>
<th>Type of therapy</th>
<th>n</th>
<th>Average age (range), yr</th>
<th>Platelet-rich plasma serotonin μg/liter ± SEM #</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-3,4-Dihydroxyphenylalanine</td>
<td>10</td>
<td>65.3 (55-80)</td>
<td>131 ± 33</td>
</tr>
<tr>
<td>“Sinemet”</td>
<td>23</td>
<td>64.2 (54-68)</td>
<td>119 ± 39</td>
</tr>
</tbody>
</table>

* The two groups are not significantly different (P > 0.05).
Table 2. Chromatographic Investigation of Potential Interferences

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time, min</th>
<th>Peak width</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amino acids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-3,4-Dihydroxyphenylalanine</td>
<td>2.0</td>
<td>0.7</td>
</tr>
<tr>
<td>L-4-Hydroxy-3-methoxyphenylalanine</td>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td>L-5-Hydroxytryptophan</td>
<td>2.2</td>
<td>1.1</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>2.2</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>Neutral compounds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,4-Dihydroxyphenethyl alcohol</td>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td>3,4-Dihydroxyphenylglycol</td>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Acidic compounds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td>5-Hydroxyindole-3-acetic acid</td>
<td>2.2</td>
<td>1.1</td>
</tr>
<tr>
<td>3,4-Dihydroxyphenethylactic acid</td>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td>3,4-Dihydroxymandelic acid</td>
<td>2.5</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Basic compounds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Norepinephrine</td>
<td>2.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Normetanephrine</td>
<td>4.0</td>
<td>2.0</td>
</tr>
<tr>
<td>3,4-Dihydroxybenzylamine (internal std.)</td>
<td>4.0</td>
<td>2.0</td>
</tr>
<tr>
<td>L-Epinephrine</td>
<td>5.2</td>
<td>2.3</td>
</tr>
<tr>
<td>Dopamine</td>
<td>6.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Serotonin (5-hydroxytryptamine)</td>
<td>9.3</td>
<td>3.8</td>
</tr>
<tr>
<td>α-Methyldopamine</td>
<td>18.2</td>
<td>7.0</td>
</tr>
<tr>
<td>Epine (N-methyldopamine)</td>
<td>18.5</td>
<td>7.0</td>
</tr>
<tr>
<td>Metanephrine</td>
<td>23.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Tryptamine</td>
<td>(24.5)*</td>
<td>(21.3)*</td>
</tr>
<tr>
<td>5-Methoxytryptamine</td>
<td>(28.0)*</td>
<td>(31.7)*</td>
</tr>
<tr>
<td>3-Methoxytyramine</td>
<td>46.0</td>
<td>17.0</td>
</tr>
<tr>
<td>4-Methoxy-3-hydroxyphenethylamine</td>
<td>68.0</td>
<td>20.0</td>
</tr>
</tbody>
</table>

* Chromatograms of these species were only obtainable by increasing the applied electrode potential to +0.94 V vs. the saturated calomel electrode. They are not detected in chromatograms run at the usual +0.00 V.

...interferences, though perplexing, do not confound results, even in abnormal cases. For a species to interfere with this analysis, it must be: (a) isolated by the solvent extraction technique outlined to allow a final concentration that is about the same as that of either the internal standard (3,4-dihydroxybenzylamine) or serotonin; (b) retained by the cation-exchange resin such that it strongly overlaps either serotonin or, less important, the internal standard; and (c) electrochemically active at the carbon-paste electrode at the chosen potential, +0.60 V vs. the saturated calomel electrode. Compounds potentially capable of providing such interference include catecholamine and indoleamine precursors and metabolites. Therefore we decided to examine four groups of these species chromatographically: (a) amino acids, (b) neutral compounds, (c) acidic compounds, and (d) basic compounds. As can be seen in Table 2, none of the amino acid, neutral, or acidic species offer any problems in the serotonin analysis because they are not significantly retained on the cation-exchange column.

However, the basic compounds are all protonated in this medium (citrate/acetate buffer, pH 5.1) and thus do interact with the cation-exchange resin. Of these, only normetanephrine and epinephrine could interfere with the internal standard. However, if one uses peak heights for quantitation, epinephrine would only offer significant interference to the internal standard if its peak height was comparable to or greater than that of dihydroxybenzylamine. Although they do overlap (resolution = 0.77), this condition would be clearly discernible through a cursory examination of the recorded chromatogram. Normetanephrine, however, is virtually isographic with dihydroxybenzylamine under the present conditions. But analyses run without added internal standard failed to detect normetanephrine at the sensitivity employed. Any major interference by normetanephrine would be easily detected, because substantial amounts of normetanephrine would cause abnormally large "internal standard" peaks to appear in the samples. Finally, interference from normetanephrine is minimized since it is only partially oxidized (15% of maximum) at the chosen potential.

Due to the possible occurrence of normetanephrine interference, however, we have investigated a second compound, α-methyldopamine, for use as an internal standard which is clearly separated from serotonin. Almost isographic with deoxyepinephrine (epinephrine), α-methyldopamine could also experience interference. But deoxyepinephrine is not normally detectable in such samples either and, in any case, would be clearly evident by simply running the suspect sample without any internal standard.

Finally, if both normetanephrine and deoxyepinephrine were to present problems (a case we cannot presently foresee, but possible nonetheless), one could, of course, abandon the outlined internal standard method and use a standard addition technique. While dihydroxybenzylamine could conceivably be disturbed by normetanephrine and α-methyldopamine could be affected by deoxyepinephrine, serotonin displays no similar interferences.

**Discussion**

This investigation explored the utility of liquid chromatography combined with electrochemical detection for the analysis of serotonin in blood samples. The agreement in values between those obtained by this research and those previously reported by others (6, 15–20) for both plasma and serum samples readily demonstrates its usefulness.

In most previous research on serotonin in blood samples fluorescence was used as the method for detection and quantitation. Native fluorescence in strong HCl solutions (7, 14, 19–21) or derivatization with either ninhydrin (22) or o-phthalaldehyde (24, 25) have both been utilized; derivatization appears to afford higher sensitivity and selectivity. Even the best methods for fluorescence, however, typically list detection limits on the order of 1 μg/liter. The present method has demonstrated a capability of quantitating as little as 10 pg of injected serotonin. Considering the typical extraction efficiency and the fraction of final HCl solution injected, we estimate a lower limit of about 100 pg for the present scheme, significantly lower than fluorometry. And we are confident this limit could easily be lowered by a straightforward extension of the technique. 5-Hydroxyindole species such as 5-hydroxyindole-3-acetic acid and 5-hydroxytryptophan exhibit native fluorescence in strong HCl solutions isomorphic to that of serotonin (20). And the derivatives show some, although less, interference from 5-hydroxyindole species (24). Additional separation before analysis must be performed to assure that these are not confounding. We know of no interferences with serotonin in the liquid chromatography scheme outlined. With fluorescence, the volumes of the initial aqueous solution, butanol, heptane, and final aqueous solution must be strictly controlled. The liquid-chromatographic technique eliminates the necessity to control these variables accurately while concurrently affording a high precision through the generally recommended use of an internal standard. In the eight successive determinations of pooled serum serotonin, the average coefficient of variation for the present assay was 6.9%.
Radiochemical methods have been used in the analysis of tissue and blood serotonin. Determination of tissue serotonin by tagging with a radiolabeled group through enzymatic reaction and subsequent isolation of the product (28) is comparable in sensitivity to the liquid-chromatographic method; however, this technique has not yet been used for blood samples. Enzymes utilized in this method, now unavailable commercially, must be routinely purified. While the liquid chromatography with electrochemical detection demands proper chromatographic retention and electrochemical activity at the selected potential, the enzymic-isotopic system counts any and all radioactive materials isolated by the rather general solvent extraction. However, this ordinarily does not pose difficulties because of the high enzymic specificity and low concentrations of potential interferences. Radioimmunoassay of blood serotonin has been recently reintroduced (27) by Kellum and Jaffe (8) and examined in some detail. This method also affords a sensitivity comparable to the present technique. But interference from 5-methoxytryptamine and, to a lesser degree, tryptamine is mentioned. Although these would not pose any problems at their normal circulating concentrations, abnormal conditions or drug administration could produce serious errors. The intra-assay coefficient of variation (9.4%) is somewhat higher than that obtained by the present method (6.9%).

A technique involving gas chromatography combined with mass spectrometry has been outlined by Cattabeni et al. (28) for the determination of tissue serotonin. Although the detection limits for it are a little less favorable than those for liquid chromatography with electrochemical detection (about 35 pg compared to 10 pg of injected serotonin), this technique is unequivocally the most selective of the existing methods because of its multiple ion monitoring capabilities (29). However, it also depends upon derivitization for quantitation, thus decreasing the potential precision, and has not been shown to be applicable to blood samples.

Two other points require consideration in comparing the present to other existing techniques: processing time and cost. With the present scheme, 30 to 60 samples can be processed in a working day, a rate comparable to both the fluorescein and the gas chromatography/mass spectrometry techniques. Radiochemical techniques could conceivably increase this throughput. Second, monetary investment, although not discussed for most new techniques developed, is certainly a central consideration in the adoption of any given method. Liquid chromatography with electrochemical detection is the least expensive of those considered: two- to six-fold less expensive than fluorescence, four- to eight-fold less expensive than radiochemical counters, and 20- to 60-fold cheaper than gas chromatography/mass spectrometry.

Thus the primary advantages of the liquid chromatography with electrochemical detection are its sensitivity, high selectivity, low cost, precision, and reliability. No current technique surpasses the sensitivity limits of liquid chromatography with electrochemical detection in serotonin determinations. There are no known interferences in the proposed method for serotonin. Liquid chromatography with electrochemical detection is by far the most inexpensive of the available techniques. Even when one expands to the dual parallel system (30), which allows approximately twice the daily sample throughput (from 30 to about 60), only a moderate cost increase is required. Liquid chromatography with electrochemical detection is the only highly sensitive method discussed which does not require derivatization, thereby providing maximal precision. Finally, we have found that the equipment requires minimal maintenance and little to no repair for years of reliable performance.

The present investigation, designed to explore the wide range of applicability of liquid chromatography with electrochemical detection to the analysis of serotonin, has yielded some additional interesting results. The samples of pooled serum and blood-bank serum were both significantly (P < 0.05) lower than those of the pre-employment normals. In both cases, it appears this may be attributed to the storage of the sample for an appreciable time at room temperature before being frozen on solid CO$_2$. The specimen of pooled sera, for example, was collected during a normal working day in the laboratory, individual samples being added to the pool as received at room temperature. The pool was then mixed at the end of the day and frozen on solid CO$_2$. Deterioration may well have occurred during this time. Similarly, the blood-bank samples could have deteriorated between collection and storage on solid CO$_2$. Additionally, the blood-bank samples were stored at only ~10°C for one to six months before use. The long-term stability after storage on solid CO$_2$, however, was verified by the repeated analyses performed on the pooled sera during 16 days.

The platelet-rich plasma samples permit a comparison between 1-3,4-dihydroxyphenylalanine and L-3,4-dihydroxyphenylalanine + amino acid decarboxylase inhibitor treatment for parkinsonian patients. Although the particular inhibitor used was intended primarily to block the peripheral decarboxylation of L-3,4-dihydroxyphenylalanine, it is also known to block decarboxylation of L-5-hydroxytryptophan, an intermediate in the biosynthesis of serotonin. We believed that such treatment could simultaneously affect peripheral serotonin metabolism. The lack of a significant difference between these two groups, however, does not support this supposition. However, the groups examined were not controlled for sex, disease progression, dosage, or sampling time. Any of these factors could well have masked any differences that may have existed. Unfortunately, we cannot yet state that the peripheral decarboxylase inhibition in parkinsonian patients does not simultaneously alter peripheral serotonin metabolism.

Nonetheless, the present investigation has shown liquid chromatography with electrochemical detection to be a highly sensitive and selective method for the analysis of serotonin in a variety of blood samples. The relatively small coefficient of variation exhibited by the method should also facilitate examining groups with only small differences.

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References


