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Simultaneous measurement of serotonin and paroxetine in rat brain microdialysate by a single-pump column-switching technique

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Abstract

Simultaneous quantitation of paroxetine and serotonin in rat brain microdialysate is presented as a means to study the neuropharmacokinetics and neuropharmacodynamics of paroxetine, a selective serotonin reuptake inhibitor. In order to achieve this objective, a single-pump column-switching technique was developed. Optimization of the mobile phase in terms of the concentration of ion-pairing agent, pH of mobile phase, temperature of the stationary phase and concentration of organic modifier was investigated and a single mobile phase was developed for both separations. The design was such that the switching valve employed column I (50 mm length) and column II (250 mm length) in series in position A. At 15.3 min, the valve was switched to position B, in which the flow of the mobile phase was directed only through the short column (column I). A flow gradient program was used to increase the flow-rate from 0.125 ml/min to 0.4 ml/min, which enabled a reduction in total analysis time to less than 20 min. The limits of detection for serotonin and paroxetine were 6 fmol and 300 fmol, respectively. The accuracy of the method demonstrated percent differences from spiked samples that were within 12.5% and the precision was found to be within 10% R.S.D.

Keywords: Serotonin; Paroxetine

1. Introduction

The measurement of time-dependent changes in paroxetine levels in rat brain microdialysate is necessary for the study of the neuropharmacokinetics of paroxetine and evaluation of the relationship between levels of paroxetine in the brain and its pharmacological activity in a dynamic system in vivo. Paroxetine is a potent selective inhibitor of serotonin neuronal reuptake now widely used in the treatment of depression [1–3]. By inhibiting the active transport mechanism of serotonin, paroxetine exerts antidepressant action through an increased concentration of the neurotransmitter at the synaptic cleft [4], which would be reflected in a rat brain microdialysate sample. Caccia et al. studied the effects of short and long term administration of paroxetine on serotonin levels in rat brain tissue [5]. Total tissue levels may mask small but important neurochemical changes related to pharmacological activity. The drug- induced changes in the tissue content of serotonin is hard to interpret in terms of neuronal release. The microdialysis technique provides an estimation of the serotonin content in the extracellular compartment of the central nervous system. Estimation of the serotonin content in this

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compartment is believed to be directly related to the concentration at the site where the neurotransmitters are released in the synaptic cleft [6]. Measurement at the synapse is not yet possible and in vivo measurements in the extracellular fluid provide the most relevant information obtainable [7]. The microdialysis technique also allows the extracellular concentration of paroxetine to be measured. There are several reasons one may attempt to measure the extracellular concentration of a drug [8]. The unbound concentration of paroxetine in the brain extracellular fluid is a determinant to pharmacological activity and the time course for the extracellular fluid concentration may differ substantially from the time course observed in the blood.

The simultaneous determination of serotonin and paroxetine in rat brain microdialysate presents a bioanalytical challenge because the ratio of the concentration of serotonin in tissue homogenate to that of extracellular fluid is approximately 1000:1 [9] and a sensitive method is required. Due to the low inherent volume of the dialysate, classical techniques such as liquid–liquid extraction and solid-phase extraction to preconcentrate the sample and improve selectivity are difficult. This places an additional burden on the analytical system since high resolution separation of serotonin from endogenous substances is also required. From Fig. 1, it can be observed that serotonin is relatively hydrophilic due to the amino and hydroxyl functional groups attached to the indole ring. Paroxetine is a substituted phenylpiperidine and is designed to be lipophilic so it can cross the blood–brain barrier. This factor increases the complexity of the separation due to the wide range of chromatographic retention times between serotonin and paroxetine. The isocratic separation of such a complex sample exhibits a trade-off of resolution for early-eluting peaks, with detection of late-eluting peaks. The approaches to solve this problem include use of gradient elution or chromatographic methods with column-switching [10–12].

Several analytical methods exist for quantitation of paroxetine in human plasma. Härtter et al. developed a column switching technique with absorbance detection in human serum [13]. Brett et al. reported a HPLC method involving derivatization with dansyl chloride and subsequent fluorescence detection [14]. Petersen et al. reported a gas chromatographic procedure [15], Knoeller et al. developed a HPLC method with UV detection [16]. All the above methods were developed for quantitation of paroxetine in human serum. Kalén et al. reported a sensitive method for serotonin in rat brain microdialysate using HPLC with fluorescence detection [17]. Sarre et al. reported electrochemical detection for quantitation of serotonin in rat brain dialysate collected from the rat hippocampus [18]. Despite the fact that electrochemical detection offers excellent sensitivity for serotonin, this mode of detection cannot be easily coupled with gradients and column-switching techniques because these approaches cause disequilibrium of the electrochemical flow cell with the mobile phase. Currently, there is no analytical method reported in the literature for simultaneous quantitation of paroxetine and serotonin in rat brain microdialysate.

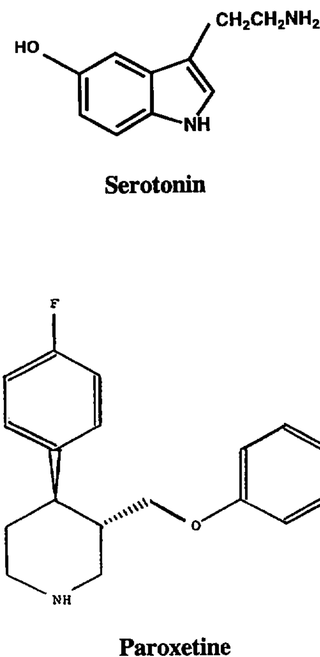

Fig. 1. Chemical structures of the analytes.

Nicholson et al. reported on the complexity of multidimensional LC systems and the difficulties involved in the development of rational optimization strategies. The stationary phase type, column temperature, mobile phase variables such as organic modifier type, concentration of organic modifier, pH, the size and concentration of an ion-pairing agent

would represent variables that would require optimization [19]. Our approach was based on data obtained from each column with respect to the variables listed above. Based on experimental observations, the mobile phase was optimized such that a single mobile phase could be used for both chromatographic systems. This is a requirement for the single-pump column-switching approach. The method that has been developed offers an alternative to conventional column-switching and the advantages include the requirements of only a single pump, a single detector and a valve switch at only one time point.

2. Experimental

2.1. Reagents

Paroxetine hydrochloride hemihydrate (87.4% free base) was supplied as a gift by SmithKline Beecham Pharmaceuticals (King of Prussia, PA, USA). Serotonin hydrochloride was purchased from Sigma (St. Louis, MO, USA). Glacial acetic acid was purchased from J.T. Baker (Phillipsburg, NJ, USA). Deionized water was distilled in glass. Acetonitrile and methanol were purchased from Baxter Healthcare (Mcgraw Park, IL, USA). The ion-pairing agent, 1-octane sulfonic acid, sodium salt was purchased from Eastman Kodak (Rochester, NY, USA). All reagents were HPLC-grade unless stated otherwise.

2.2. Spectral characterization

The spectral properties of serotonin and paroxetine were determined using a Perkin-Elmer Model LS-50 scanning luminescence spectrometer equipped with a xenon excitation source (Perkin-Elmer, Rockville, MD, USA). The excitation maxima for the analytes in the mobile phase were determined by scanning from 200 nm to 500 nm with the emission monochromator set to zero. The scan speed was set at 100 nm/s and the band width used was 10 nm. The emission spectrum was scanned after setting the excitation monochromator to the maximum wavelength obtained from the excitation scan.

2.3. Chromatography

The chromatographic system consisted of a Hewlett-Packard Model 1050 pump (Hewlett-Packard, Avondale, PA, USA), Autochrome Model 401 six-port switching valve (Autochrom, Milford, MA, USA) equipped with a Model 201 solenoid interface and a Rheodyne Model 7161 injector equipped with a 10-μl sample loop. Column I was a Nucleosil C_{18} (50×2.0 mm I.D., particle size 3 μm, Phenomenex, Torrence, CA, USA). Column II was a Supelcosil LC-18-DB (250×2.1 mm I.D., particle size 5 μm, Supelco, PA, USA). Chromatography was performed at ambient temperature. Data were acquired on a Hewlett-Packard integrator, Model 3396 A. Valve switching and the flow gradient were controlled by contact closures on the injector.

2.4. Mobile phase preparation

The optimal mobile phase used for separation consisted of 0.23 m*M* 1-octanesulfonic acid sodium salt in acetic acid (65 m*M*, adjusted to pH 2.8 with glacial acetic acid)–acetonitrile (67:33, v/v). The mobile phase was finally filtered through a 0.22-μm nylon filter and degassed by sparging with helium.

2.5. Fluorescence detection

Excitation and emission wavelengths were centered with an average excitation wavelength of 280 nm and an emission wavelength of 340 nm. The excitation bandwidth was set at 18 nm and the emission bandwidth at 40 nm. The detector gain was set at 100 and the time constant at 2 s for detection of serotonin. A Waters 474 scanning fluorescence detector (Waters, Milford, MA, USA) was time-programmed so that the gain was set to 10 from 100 at 15.3 min in order to obtain paroxetine peaks on-scale for the chromatographic system. After the column switch, the detector was programmed to perform an autozero at 15.5 min to stabilize the baseline. The autozero was performed to reset the baseline after the column-switch which caused a negative deflection in the detector due to pressure changes associated with the column-switch.

2.6. Flow gradient programming

At position A of the switching valve, the mobile phase flow-rate was maintained at 0.125 ml/min. After elution of the serotonin peak, the flow of the mobile phase was increased stepwise at 15.3 min from 0.125 ml/min to 0.4 ml/min at 15.4 min by programming a timed-flow gradient with the HP Model 1050 pump.

2.7. Column-switching

The switching valve was pneumatically activated and its position was controlled by use of the system controller, interfaced to the switching valve by a solenoid interface. The switching valve positions used during the analysis are also shown.

2.8. Microdialysis studies

Male Sprague–Dawley rats (250–350 g) were housed under diurnal lighting. Animals were given ad libitum access to food and water. Rats pretreated with atropine (1 mg/kg, intraperitoneally) were anaesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally). The head was shaved, treated with Betadine and placed in a stereotaxic device. A small hole was drilled through the skull and an intracerebral guide cannulae (CMA/12, BAS, West Lafayette, IN, USA) was inserted into the anterior lateral striatum. The spatial coordinates were 2 mm anterior to the bregma, 3 mm from the mid-sagittal suture and 6 mm ventral from the dura [20]. After implantation the cannula was fixed firmly to the skull with anchor screws and dental cement and occluded with a obdurator. The dialysis experiments, were performed at least 3 days after surgery to allow the animals to recover. On the day of the experiment, the obdurator was removed and the microdialysis probe (CMA/12 probe, 3 mm length, BAS) was inserted into the striatum via the guide cannulae and perfused continuously with perfusion fluid (Na^+ 147 m*M*, K^+ 3.5 m*M*, Ca^{2+} 1.0 m*M*, Mg^{2+} 1.2 m*M*, Cl^- 129 m*M*, Po_4^{3-} 1 m*M*, HCO^- 25 m*M*) via polyvinyl tubing attached to the implant on the rat's head with a microperfusion pump (CMA 100, Carnegie Medicin, Sweden) at a flow-rate of 0.75 μl/min. The baseline stabilization occurred 2 h following probe insertion. For the brain infusion, paroxetine hydrochloride hemihydrate was dissolved in the perfusion fluid at a concentration of 0.05 μ*M* and infused through the probe at a flow-rate of 0.75 μl/min for 30 min. Fractions of the dialysis solution were collected over a period of 30 min with an automated fraction collector. The animals were placed in a plexiglass container during the experiment.

3. Results and discussion

3.1. Separation

The rat brain microdialysate matrix consisted of endogenous substances and their metabolites. Neurotransmitters such as serotonin are very polar and their separation is complicated by shortened analysis time relative to other analytes, poor resolution and inadequate peak symmetry due to secondary stationary phase interactions caused by residual silanol groups [21]. Ion-pair chromatography can be used to enhance the retention selectivity of analytes like serotonin and paroxetine, which possess ionizable amine functional groups by addition of a negatively charged ion-pair to the mobile phase. Separation conditions for simultaneous quantitation of serotonin and paroxetine were investigated by evaluation of the effects of the concentration of the ion-pairing agent, pH of the mobile phase, the concentration of organic modifier, flow gradient programming, temperature of the column and the single-pump column-switching technique.

3.2. Chromatographic data obtained from system I and system II

The analytical column for system I consisted of a Nucleosil C_{18} (50×2.0 mm I.D.) reversed-phase column. The analytical column used for system II was a Supelcosil LC-18 DB (250×2.1 mm I.D.) column.

3.2.1. Effect of ion-pairing agent

The dependence of capacity factor (k') on the concentration of the counter-ion was studied with a mobile phase containing acetic acid (65 m*M*, adjusted to pH 2.8 with glacial acetic acid)–acetonitrile

(67:33, v/v). The counter ion used was 1-octanesulphonic acid, sodium salt. The concentration range studied was from 0.15 m*M* to 0.77 m*M*. No effect of the counter-ion concentration on capacity factor was observed. The concentration dependency of k' on ion-pairing agent, the concentration would likely have been observed if the concentration range had been greater. Observation from experimental data suggests that at concentrations above 0.15 m*M* of the ion-pairing agent the capacity factors for serotonin and paroxetine maximized at 1.7 and 17.4, respectively. A lower concentration of the ion-pairing agent would have decreased the capacity factors for all the analytes and the k' for serotonin would have been less than 1.7. This would cause elution of serotonin at the solvent front and because of this, a lower concentration range was not studied. The results obtained for the system II were similar to that obtained with system I. The retention times of the analytes were unaffected in the concentration range studied for both system I and system II. This experimental observation demonstrates the ruggedness of the system and was exploited to develop single mobile phase for the single-pump column switching approach.

3.2.2. Effect of pH

The dependence of capacity factor on pH of the mobile phase was studied with a mobile phase containing 0.23 m*M* octane sulfonic acid, sodium salt in acetic acid (65 m*M*, pH 2.8 adjusted with glacial acetic acid)–acetonitrile (67:33, v/v). In the pH range studied, from 3 to 7, no effect of pH on capacity factor was observed. The amino group in serotonin has a pK_a of approximately 9.8 and the piperidine group in paroxetine has a pK_a of approximately 10.3. In the pH range studied, both analytes are completely ionized, and pH greater than 7 was not investigated since silica based columns degrade above this pH [22]. The results obtained with chromatographic system II were similar to that with system I and can be explained as above with chromatographic system I.

3.2.3. Effect of temperature

The effect of temperature on k' was studied in the range of 25°C to 45°C. Throughout the temperature range studied, the capacity factors for all analytes remained unchanged. Temperature influences the behavior of a solute partitioning between the stationary phase and the mobile phase. A temperature increase will, generally be associated with a decrease in k' [23]. The effect of temperature was studied with a mobile phase at pH 3, at this pH serotonin and paroxetine are completely ionized. A greater temperature range was not studied since higher temperatures may reduce the life of a bonded hydrocarbon to silica type packing [22].

3.2.4. Effect of the organic modifier concentration on chromatographic system I

The effect of increasing the concentration of acetonitrile in the mobile phase was evaluated. An acetonitrile concentration of 33% was found to be optimal for elution of serotonin at 1.8 min and paroxetine at 17.5 min using a flow-rate of 0.2 ml/min. Increasing the concentration of acetonitrile from 33% to 38% resulted in elution of paroxetine at 11 min; but, at this higher concentration, serotonin eluted with the solvent front. The final chromatographic conditions employed with chromatographic system I consisted of a Nucleosil C_{18} column with a mobile phase containing 0.23 m*M* octane sulfonic acid, sodium salt in acetic acid (65 m*M*, pH 2.8 adjusted with glacial acetic acid)–acetonitrile (67:33, v/v). The analysis times for serotonin and paroxetine were 1.8 min and 17.5 min, respectively at a flow-rate of 0.2 ml/min. This method was considered to be optimal for blank matrix standards. Serotonin was unresolved from early eluting neurochemicals originating from the matrix when rat brain microdialysate samples were injected. This can be observed in Fig. 2. The perfused rat brain microdialysate showed an absence of interfering peaks at the retention time of paroxetine.

3.2.5. Effect of organic modifier concentration on chromatographic system II

The resolution of serotonin from interferences was optimized by using acetonitrile as an organic modifier in the concentration range from 20% to 40%. At an acetonitrile concentration of 40% serotonin remained unresolved from the interferences in the rat brain microdialysate matrix (see Fig. 3). When the concentration of acetonitrile was 20%, serotonin was resolved, but the analysis time was increased to 17

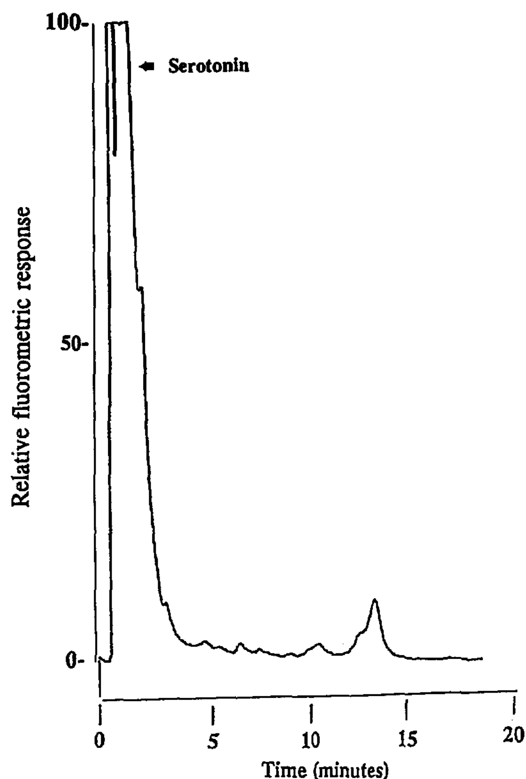

Fig. 2. Chromatogram from rat brain microdialysate, illustrating a lack of resolution of serotonin from interfering substances on system I.

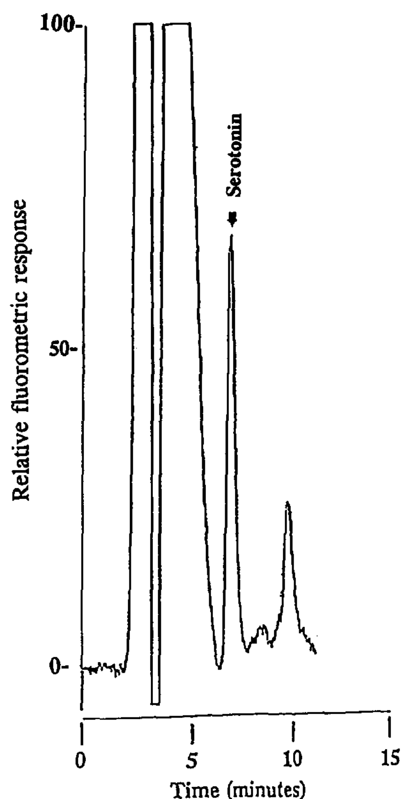

Fig. 3. Chromatogram from rat brain microdialysate, illustrating optimal resolution of serotonin from interfering substances on system II.

min for serotonin and 95 min for paroxetine. At a concentration of 33%, serotonin was optimally resolved with an analysis time of 7 min and the retention time of paroxetine was 75 min at a flow-rate of 0.2 ml/min. Based on the above results an acetonitrile concentration of 33% was chosen for further optimization experiments.

The use of gradient elution was considered as a means to resolve serotonin from the interfering substances. We observed, however, baseline drift and a reequilibration time of 30 min between successive injections when a gradient was used [24]. The use of a gradient was therefore not evaluated further based on these observations.

3.2.6. Evaluation of flow gradient programming

The retention time for serotonin on system II was 7 min, and serotonin could be resolved from potentially interfering substances on Chromatographic system II. However, the retention time for paroxetine on this system was 75 min. A flow gradient programming in which the flow-rate was maintained at 0.2 ml/min for 7 min until elution of the serotonin peak and then increased to 0.4 ml/min reduced the analysis time of paroxetine from 75 min to 52 min. The above chromatographic system with column II was optimal for serotonin but inappropriate for simultaneous analysis because of the unreasonably long analysis time for paroxetine of 52 min even with the use of a flow gradient.

3.2.7. Coupling of column I and column II in series

The following experiment was designed to further evaluate the feasibility of the single-pump column-switching technique. Coupling column I and column II in series would theoretically result in an analysis

time which would be additive. On column I, the analysis time for serotonin was 1.8 min and the analysis time on column II was 7 min. It was observed that connecting the columns in series resulted in an analysis time for serotonin of 8.9 min. Connecting the columns in series was optimal for resolution of serotonin but for paroxetine the analysis time was 92.5 min. Elution of paroxetine would, therefore, require a configuration in which only column II was utilized.

3.3. Single-pump column-switching technique

Column I was optimal for separation of paroxetine in terms of the analysis time but inappropriate for resolving serotonin from interfering substances. Column II was optimal for resolving serotonin but inadequate for paroxetine due to long retention time on this system. The use of the short column (column I) resulted in a lower k' for the late-eluting components and the use of the longer column (column II) resulted in increased resolution for early-eluting components. Coupling these two columns via a column-switching approach provides a strategy for optimization of the separation. The classical approach would be to use conventional heart-cut column-switching which would require the use of two pumps and two valves [10]. In order to simplify the system, a design using a single pump, single valve and a single detector was developed. Fig. 4a represents a schematic diagram of the chromatographic system with single-pump column switching. Fig. 4b represents the different positions of the switching valve. The design makes use of a Autochrome Model 401 six-port switching valve. Column I was connected to position 6 of the switching valve and position 4 and 5 were connected by tubing (0.007 in. (1 in.=2.54 cm) I.D). Column II was connected between position 1 and 2 of the six-port switching valve. Position 3 of the switching valve was connected to the fluorescence detector. The configuration was such that the flow of mobile phase was through column I and column II and finally to the detector in position A, which provides the high resolution necessary for separation of serotonin from interfering substances. After elution of serotonin the valve is switched to position B, in which the flow of mobile phase is through column I, the loop of the switching valve and finally to the detector. This configuration enabled earlier elution of paroxetine with flow gradient programming. The procedure was implemented by strategic optimization of the mobile phase such that a single mobile phase was suitable for both systems. This is the primary condition necessary for the system to function. Based on the optimization data obtained for system I and II, a single mobile phase consisting of 0.23 mM 1-octanesulfonic acid sodium salt in acetic acid (65 mM, adjusted to pH 2.8 with glacial acetic acid)–acetonitrile (67:33, v/v) was chosen.

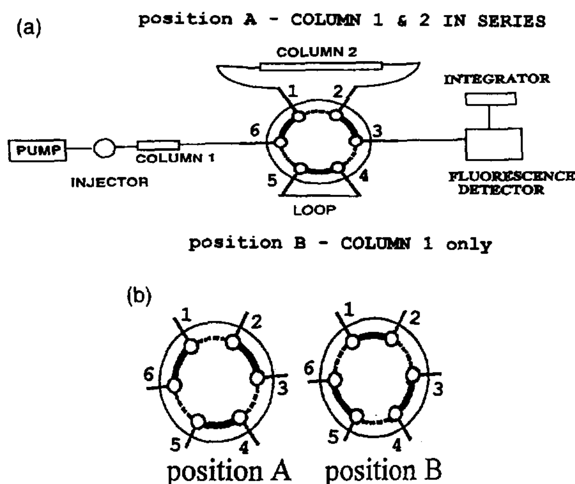

Fig. 4. (a) Schematic diagram of chromatographic system with single-pump column-switching. (b) Position A: flow through column 1 and 2. Position B: flow through column 1 only.

3.4. Optimization of chromatographic conditions with the single-pump column-switching technique

The final separation conditions were optimized by varying the flow gradient program and the column-switching time. Initially, a flow gradient program was used in which the flow-rate was 0.1 ml/min until the elution of serotonin at 17.1 min and the flow-rate was then increased to 0.3 ml/min. Using this program, the elution time for serotonin was 17.1 min and for paroxetine was 25 min. The flow program was modified to reduce the analysis time without compromising resolution to an initial flow-rate of 0.125 ml/min with a switch to 0.4 ml/min after elution of serotonin at 13.7 min. The analysis

time could then be reduced to less than 20 min. The column-switching time was determined by observation of the elution time of the serotonin peak. Coupling columns I and II in series resulted in an analysis time of 13.7 min for serotonin. The column-switching time was set at 15.3 min to allow complete elution of the serotonin peak.

3.5. Selectivity

The selectivity of the method was studied by analysis of microdialysate samples obtained from untreated rats. Serotonin is an endogenous neurotransmitter, and a serotonin peak is expected in the blank obtained from the rat brain. Structural confirmation of serotonin was attempted with electrospray mass spectroscopy after fraction collection of the eluent corresponding to the retention time window for serotonin. This attempt proved to be futile due to the trace amounts in the fmol range for serotonin. Spectral scanning with the fluorescence detector for endogenous serotonin was also found to be unsuitable for spectral identification due to the low fmol range of serotonin. The serotonin peak was identified on the basis of chromatographic retention time by comparison with a reference standard using fluorescence detection which offers selectivity. Endogenous interference was observed for paroxetine from samples collected from the anterior lateral striatum, as can be observed from Fig. 5. Fig. 6 shows the levels of serotonin and paroxethine in rat brain microdisylate from the anterior lateral striatum following brain infusion of paroxetine (see also Section 3.9). In samples collected from rat brain cortex, no endogenous interference was observed as can be observed from Fig. 7. It would be useful to study the effect of paroxetine on serotonin levels in rat brain striatum. Subtraction of the peak height obtained from the blank was used for quantitation of paroxetine in samples collected from striatum. Studies were carried out using samples from rat brain striatum to validate the blank subtraction approach.

3.6. Calibration

The matrix used to prepare standards in order to generate the calibration curves for quantitation consisted of blank microdialysate. The calibration curve

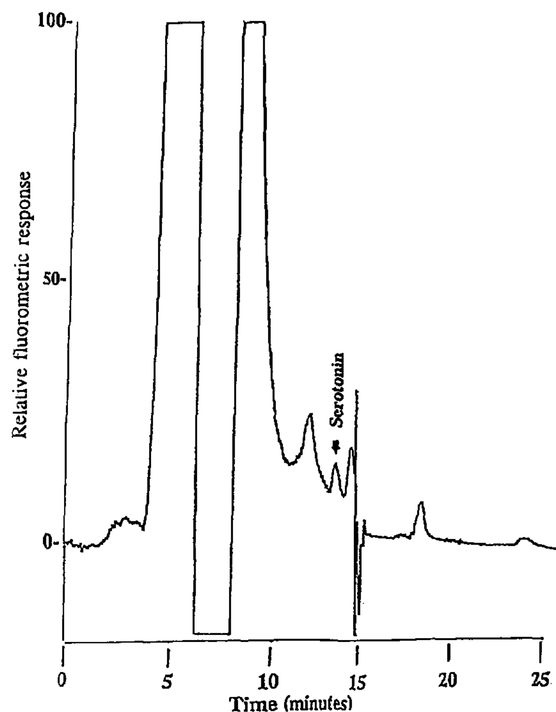

Fig. 5. Chromatogram of rat brain microdialysate from anterior lateral striatum obtained prior to paroxetine treatment, representing 20 fmol of baseline serotonin.

for serotonin was found to be linear throughout the range of 0.015–0.306 pmol which was established as the range of interest. The mean correlation coefficient for the calibration curves tested ($n=3$) was 0.993. A plot of log concentration versus log response demonstrated a slope of 1.01 which further validates linearity [25]. The calibration curve for paroxetine was found to be linear from 4.2 pmol to 58 pmol on column. The mean correlation coefficient of the paroxetine calibration curves ($n=3$) was 0.997. A plot of log concentration versus log response demonstrated a slope of 1.03.

3.7. Detectability

The quantitation was accomplished using the external standard method. The limit of detection of the analytes was calculated at a signal to noise ratio of 3. The LOD of serotonin was 6 fmol and for paroxetine was 300 fmol. The LOD of serotonin and paroxetine were determined by setting the gain in the

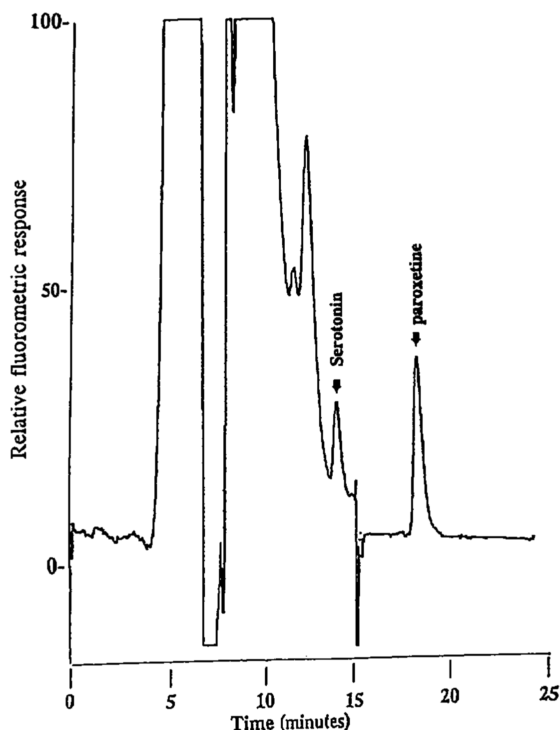

Fig. 6. Chromatogram of rat brain microdialysate from anterior lateral striatum after brain infusion of paroxetine. The levels of serotonin and paroxetine represented are 49 fmol and 16.3 pmol, respectively.

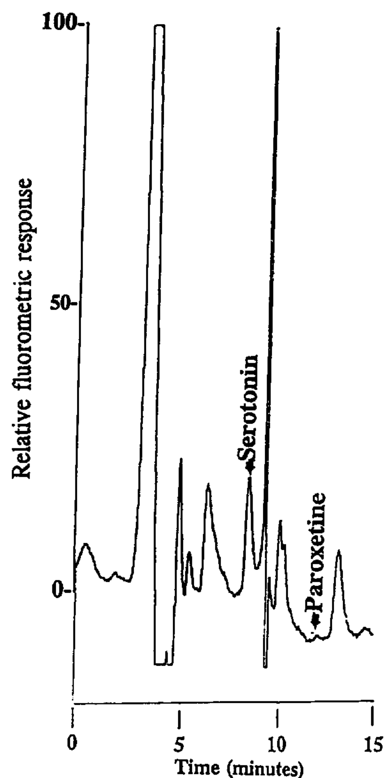

Fig. 7. Chromatogram from rat brain cortex, illustrating selectivity of the method for paroxetine.

detector at 100. The LOQ for serotonin at *S/N* of 10 was determined to be 21.3 fmol on column. The gain was set at 10 in order to obtain paroxetine peaks on scale. The LOD for paroxetine at a gain of 10 was 1.2 pmol and the LOQ obtained was 4.2 pmol on column.

3.8. Accuracy and precision

The accuracy of the method was evaluated by calculation of the percent difference of assayed values from spiked control concentrations and precision is indicated as the %R.S.D. for these spiked controls. Accuracy and precision of the method for serotonin and paroxetine is shown in Table 1. The accuracy of the method was within 12.5% difference from the spiked value and the precision was within 10% R.S.D..

3.9. Application of the method in rat brain

Fig. 5 shows a chromatogram of blank dialysate obtained from rat brain anterior lateral striatum prior to administration of paroxetine. Fig. 6 shows a chromatogram obtained from a sample collected after infusion of paroxetine. The chromatograph represents 16.3 pmol of paroxetine. It can be observed from the chromatograms that paroxetine causes an increase in the levels of serotonin from 20 fmol to 49 fmol.

4. Conclusion

A single-pump column-switching technique in conjunction with flow gradient programming and fluorescence detection has been developed for simultaneous quantitation of serotonin and parox-

Table 1
Precision and accuracy of the method for serotonin and paroxetine ($n=6$)

Analyte	Spiked concentration (pmol/10 μl)	Mean assayed concentration (pmol/10 μl)	R.S.D. (%)	Accuracy (%)
Serotonin	0.016	0.018	8.0	12.5
	0.060	0.064	5.9	6.6
	0.283	0.270	3.8	4.8
Paroxetine	4.5	4.1	8.3	8.8
	17.3	16.5	6.4	4.6
	50.2	46.5	5.1	7.3

etine in rat brain microdialysate. Chromatographic variables for both the columns were evaluated and optimized such that a single mobile phase was optimal for both systems. The method achieved selectivity for serotonin with regard to interfering substances in rat brain microdialysate with concurrent elution of paroxetine in a reasonable analysis time of less than 20 min. The method was used for simultaneous monitoring of the concentration of paroxetine and for low level quantitation of serotonin in rat brain microdialysate in vivo. The potential of the method for studying the neuropharmacokinetics of paroxetine with the neuropharmacodynamic activity on serotonin levels in vivo is demonstrated in rat brain using microdialysis.

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