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Review

High-performance liquid chromatographic analysis with electrochemical detection of biogenic amines using microbore columns

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Abstract

High-performance liquid chromatography with electrochemical detection (HPLC–ED) is a popular method for measuring biogenic amines, owing to its simplicity, versatility, sensitivity, and specificity. Recent developments in microbore column HPLC–ED have been facilitated by miniaturization of solvent delivery, column packing, sample injection and micro-flow cell construction. The aim of this paper is to present an overview of recent developments in microbore column HPLC–ED, in terms of advantages and limitations. This paper covers the recent advancements and important factors of HPLC–ED analysis of biogenic amines using microbore columns. Particular emphasis is placed on applying this technique to microdialysis, for which great sensitivity is required. Its potential in future biomedical applications is also discussed.

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1. Introduction

1.1. Biogenic amines and related compounds

Biogenic amines, including catecholamines and indoleamines, play a number of important functions in the peripheral and central nervous systems. Levels of biogenic amines and their metabolites in tissues or biological fluids have been widely investigated for a variety of physiological and disease states. These compounds include parent neurotransmitters, norepinephrine (NE), epinephrine (E), dopamine (DA), serotonin (5-HT), their precursors, 3,4-dihydroxyphenylalanine (DOPA), 5-hydroxytryptophan (5-HTP), their major metabolites, 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), vanillylmandelic acid (VMA), 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA).

These biogenic amines and their related compounds have been implicated in the neurochemistry and physiology of mental illnesses and neurological disorders. 5-HT is involved in depression [1,2], schizophrenia [3,4], migraine [5], carcinoid [6,7], mental retardation [8,9], infantile autism [10], body temperature regulation [11], sensory perception [12], and sleep regulation [13]. NE, E and DA are involved in depression [14], schizophrenia [15], Parkinsonism [16,17], essential hypertension [18,19], pheochromocytoma [20,21], cardiac arrhythmia [22,23] and melanoma [24,25]. Therefore, for clinical diagnosis or prognosis, and for basic research purposes, it is necessary to determine concomitant levels of serotonin, catecholamines and their metabolites in tissues and biological fluids, including urine, blood and cerebrospinal fluid. The complexity of biological matrices and the diverse levels of biogenic amines, their precursors and their metabolites requires the use of an efficient separation technique and sensitive detection devices.

1.2. Development of analytical techniques

Many analytical techniques, such as radiochemistry [26,27], gas chromatography [28,29] and liquid chromatography [30,31], have been developed for the determination of biogenic amines, their precursors and metabolites. Many excellent reviews [32–35] and textbooks [36–38] have been published, covering techniques, theories and applications in detail.

High-performance liquid chromatography with electrochemical detection (HPLC–ED) is considered to be one of the most popular methods for determining biogenic amines, owing to its simplicity, versatility, sensitivity and specificity. HPLC–ED was pioneered by Kissinger et al. [39] two decades ago. In their design, the column effluent passes in a thin-layer flow cell over the surface of a glassy carbon electrode, and parts of substances are oxidized at the electrode surface. The principle of electrochemical detection is based on the measurement of anodic current at a controlled potential applied to the electrode as a function of substances oxidized.

Many assays that have utilized HPLC-ED for the analysis of urine, blood, tissue, cerebrospinal fluid and discrete brain tissue have been published [40–48]. The detection limit for biogenic amines, using conventional HPLC-ED techniques, is about 30 fmol. This is often sufficient for determinations of biogenic amines in body fluids. Nevertheless, some of the biogenic amines in body fluids are below the detection limit of conventional HPLC-ED systems, in particular, biogenic amines in dialysates, collected by the microdialysis technique. In order to accommodate the low levels of biogenic amines, continued efforts have been made to improve HPLC-ED

methodology. For example, HPLC miniaturization has been subjected to intensive development to achieve optimum detection limits and to overcome the above problems. In addition, developments in column packing technology, reliable electrochemical detectors and simplified sample clean-up procedures have facilitated steady and encouraging progress in the miniaturization of HPLC technology.

Miniaturization initiated the renaissance of microbore column HPLC in the separation of nucleotides performed by Horvath *et al.* [49] in 1967. Microbore columns (1.0 mm I.D.) were then applied to the separation of urinary catecholamines and their metabolites by Hirata *et al.* [50] in 1980. Most recently, successful applications of microbore columns in the measurement of biogenic amines and their metabolites have been described [51,52]. The importance of small internal diameter chromatographic columns for LC has been demonstrated [53–55]. Compared with conventional analytical columns of 3.0–4.6 mm I.D., a microbore column of 1.0–2.0 mm I.D. leads to an increase in detection sensitivity for biogenic amines and reduces detection limits from picomolar to femtomolar levels (as shown in Figs. 1 and 2) [56–58].

The term "microbore" is somewhat a misnomer, as it is commonly used to describe columns of 0.5–1.0 mm I.D. and conventional lengths (10–15 cm). The small internal diameter and slow flow-rate offer a significant signal enhancement in terms of reduced elution volume and noise. Therefore, microbore columns are able to facilitate the separation and determination of samples with extremely small volumes and/or low concentrations of analytes [59,60]. Recently, femtomolar or even sub-femtomolar detection limits for biogenic amines have been achieved in the analyses of extremely small pieces of tissues and small volumes of body fluids [61,62].

Developments in microbore HPLC have been facilitated by miniaturization of solvent delivery [63,64], column packing [65–67], sample injection [68,69] and micro-flow cell construction [70,71]. Although these factors make microbore HPLC techniques slow, the ability to work with a small sample volume and small flow cell has been improved dramatically in the past decade [72,73]. Microbore HPLC–ED analysis has resulted in steady, encouraging progress that has led to sub-femtomole per injection detection limits for biogenic amines [53–58,74–76].

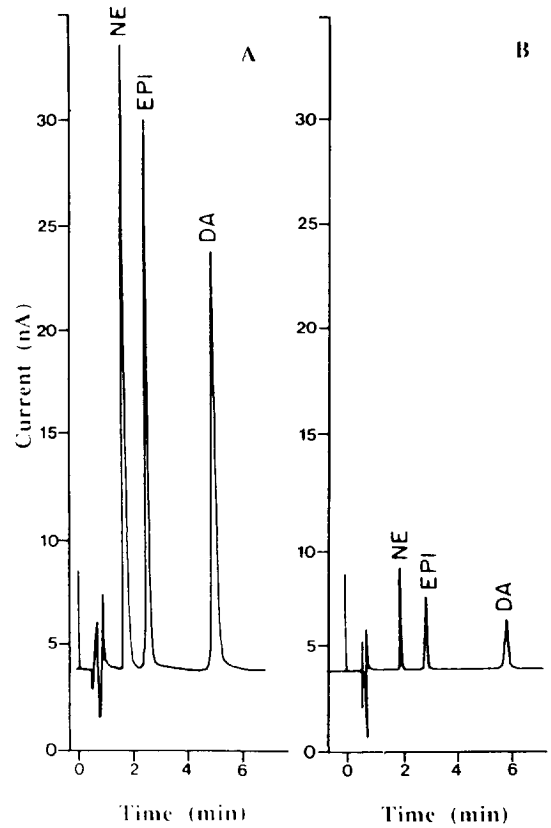

Fig. 1. Comparison of microbore and conventional columns. Sample loop (5 μl) with same amount of catecholamines (0.5 ng) injected on to the two columns, (A) BAS microbore ODS column (100 × 1.0 mm I.D., 3 μm), flow-rte 70 μl/min, and (B) conventional ODS column (100 × 3.2 mm I.D., 3 μm), flow-rate 0.7 ml/min. (From Ref. [80].)

The aims of this paper are to present an overview and to describe the recent developments in microbore HPLC methodology, in terms of advantages and limitations. Emphasis has been placed on the practice of microbore column HPLC–ED and its potential in future biomedical applications.

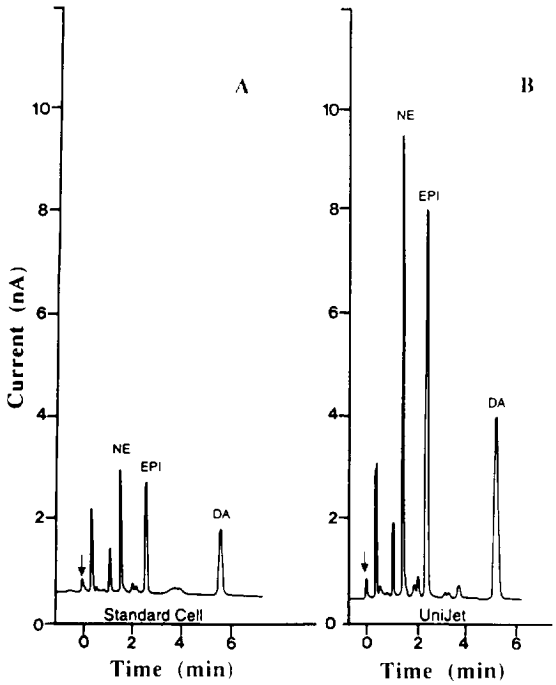

Fig. 2. Improvement in response to the UniJet detector cell (right) over the classical BAS thin-layer cell (left) under microbore conditions (100 μl/min) with all other conditions held constant; 0.5 μl of 15 pg catecholamines injected. (From Ref. [107].)

2. Microbore HPLC–ED practice

2.1. Solvent-delivery systems

A variety of commercially available pumps can be applied to microbore column HPLC. Syringe pumps are pulse-free and ideal for working with microbore HPLC. Otherwise, pulseless solvent delivery can be achieved either by the use of a conventional pumping system with micropump head(s) or by mechanical (or electronic) compensation in a conventional pump to eliminate pulses (<1%). The design of a steady solvent delivery system is particularly challenging in microbore column HPLC owing to the very low flow-rates. For instance, a microbore column of 0.5 mm I.D. requires a flow-rate range between 10 and 30 μl/min. This flow-rate range can be delivered without difficulty by syringe pumps or by some commercially available pumps with very

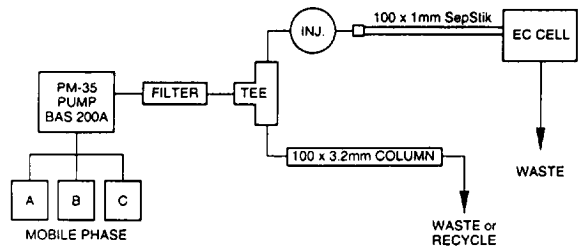

Fig. 3. Schematic diagram of flow splitting in HPLC–ED using the BAS 200A system. Both columns should be contained within the oven for the sake of precision. (From Ref. [77]).

low pulsation. Fortunately, most of these pumps function well in the laboratory. Alternatively, a flow splitting method has been described (Fig. 3) for providing the low flow-rates required for microbore HPLC in conventional HPLC pump systems [77]. Instead of using a micropump or syringe pump, a conventional HPLC pump can be connected to a T-shaped split to which two columns (one microbore and one analytical column) are connected in parallel. For both microbore and conventional columns, linear velocities in the mobile phase are approximately the same, with an overall mobile phase flow-rate of 0.5–1.5 ml/min. However, the true flow-rate of the microbore column can be adjusted to 50–100 μl/min according to differences in the total flow-rates and the columns' cross-sectional areas. The reproducibility of the flow-rates and retention capacities of analytes are consistent for microdialysates and other clean samples [78]. However, this splitting technique is cumbersome when dealing with tissues or other untreated body fluids, which may clot the microbore columns and produce irregular flow-rates or alter the retention behaviour of eluates.

2.2. Mobile phases

Several approaches for manipulating chromatographic separations of biogenic amines and their related compounds have been reported [35,36,79,80]. In general, an isocratic separation mode has been adopted for most HPLC–ED biogenic amine studies. A gradient elution sys-

tem is possible in conventional HPLC–ED, whereas gradient elution in microbore HPLC–ED may cause significant baseline drift at high detection gain, fluctuating background noise and difficulty in maintaining accurate flow-rates. In addition, the mixer volume and connections should be minimized to avoid distortion in the response time. Some excellent studies concerning these gradient problems can be found in the literature [81,82].

Occasionally, a number of electroactive interferences (substances) with diverse concentrations, present in the samples, may cause problems in chromatographic separation. They often obscure the peaks of interest. Therefore, a modification of the mobile phase is often necessary to optimize the separation of the analytes. Many optimum mobile phase compositions have been developed for conventional HPLC–ED [83,84]. The effects of the mobile phase described for conventional columns should be similar to those of microbore columns with the same type of packing material. Theoretically, if an appropriate separation can be obtained on a conventional column, it should also be obtained on a microbore column with the same mobile phase.

Changes in the pH of the mobile phase produce changes in the retention behaviour of each peak based on the charges of the analytes. The concentration of ion-pairing agents is one of the main variables affecting the retention of analytes. In general, there are no problems in separating dopamine and/or serotonin, which are eluted sequentially from a reversed-phase C_{18} column. For some weakly retained compounds, such as NE, E, DOPA, VMA and MHPG, separation may be difficult, leading to false assignment or inaccurate measurement, owing to co-elution of interferences. Modification of the mobile phase, pH values, ion-pairing agents or organic modifiers, to optimize the separation of these compounds, is frequently needed. The relative retention behaviours of a variety of biogenic amines have been reported in detail [83,84]. Three major mobile phase compositions with phosphate, citrate and monochloroacetate buffer systems have been well documented in the separation of biogenic amines and related compounds [85].

2.3. Injection and connection

Dead volumes in the injector, microbore column and detector connections can severely degrade chromatographic resolution in microbore HPLC. Thus, the connections and injection systems for microbore HPLC must be carefully considered [86,87]. A short stainless-steel capillary sample transfer tube for sample introduction has been employed by Ishii *et al.* [88]. A syringe injection split injector, which allows a small sample volume to be injected on to a microbore HPLC, has been investigated by Tsuda and Nakagawa [89]. A syringe needle split injector providing satisfactory quantitative reproducibility has been reported by Yang [90]. Direct injection of a small sample, of 70 nl, via an internal loop is now available from Valco Instruments (Houston, TX, USA). Direct injection of 1–10 μl samples on to typical microbore columns (100 × 1.0 mm I.D.) is possible for sample solutions with a relatively strong solvent mobile phase and/or a relatively weak solvent strength.

The ratios of the microbore column dead volumes to the system volume play an important role in overall LC system performance. In order to minimize the injector dead volumes, a Rheodyne Model 8125 injector can be used in a microbore HPLC–ED system instead of the more common Rheodyne Model 7125 injector [91]. The diameters of the flow channels of the Model 7125 are 0.46 and 0.61 mm and that in the Model 8125 is 0.20 mm. Therefore, the dispersion of the Model 8125 is one fifth that of the Model 7125. These injection systems ensure reproducible injection volumes, eliminating one possible source of error. Some types of conventional injection valves have a greater area of dead volume than others. Hence the injector and injection valves must be selected with care.

Other dead-volume areas include those produced between the column and the injection valve or the detector. Microbore columns can be connected to the injector or cell using fused-silica tubing and zero dead-volume fitting unions.

Reducing the number of connections or the length of connecting tubing also minimizes peak broadening [92]. The BAS SepStik (Bioanalytical Systems, West Lafayette, IN, USA) is a new type of microbore column, constructed from 1.59 mm O.D. steel tubing. It can be directly connected to a sample injection valve and/or an electrochemical cell without additional connecting tubing or unions, thus minimizing the column and system dead volumes [77].

2.4. Microbore column technology

Much of the interest in microbore HPLC has been the result of increased mass sensitivity and lower solvent consumption. In order to minimize the extra-column effects, more attention must be paid to pumps, injection valves (or autosampler), connecting tubings, column hardware and flow cells. Although there are many manufacturers of microbore columns, most columns are still prepared manually in analytical laboratories. Chromatographers can achieve sufficient sensitivity and efficiency to acquire data, at a reasonable cost, by packing their own microbore columns. Columns can be easily repacked in order to achieve economical efficiency. Analytical practice has demonstrated the difficulty in reducing the system dead volume in microbore HPLC. A better injector or an autosampler with narrower connecting tubing must be designed at the expense of lower sample volume and mass sensitivity. Indeed, shorter connecting tubing results in satisfactory data, owing to increased mass sensitivity and reduced peak dispersion.

An electrochemical detection unit seems more suitable for coupling to a microbore HPLC system than any other conventional detector. In general, an amperometric flow-cell detector is easily adapted to sample zones below 100 nl. In addition, with an electrochemical detector, more time is available for the analytes to react at the electrode at a decreased flow-rate, hence the coulometric yield will increase. The lower flow-rates also provide signal enhancement. In fact, commercial amperometric detectors are readily adapted to microbore HPLC.

Microbore column-packing procedures have been studied by many investigators [65–67,93]. The quality factors of the packing material include base silicas, particle shape (spherical or irregular), particle size (3–10 μm), surface coverage, end-capping and monomeric or polymeric phases. In addition, conditioning, drying and activation of an appropriate packing material have been extensively described [94,95]. In fact, there have been studies concerning difficulties in reproducing complex separations when changing batches of C_{18} packing material or packing procedures [65,66]. A good rule to follow is to pack a number of microbore columns by appropriate slurry packing procedures. In general, slurry made of 1–5% C_{18} packing material, in 1–2 ml of acetone (or 2-propanol or methanol), is pressed and filtered off in the column, followed by passing 10–20 ml of acetone (or 2-propanol or methanol) through the column. After 10–20 ml of solution have passed through the column, and after all pressure has been relieved from the lines, the column packing procedure is completed. A 0.5–1-mm frit is inserted into the end of the column. Microbore columns can be packed at very high pressure (400–1000 MPa). The general method described above provides good microbore columns (0.3–1.0 mm I.D.) with a range of 10 000–50 000 theoretical plates per metre compared to 5000–10 000 theoretical plates per meter in conventional HPLC [93]. Detailed procedures for packing microbore columns have been described [65–67,96–98]. The use of microbore columns allows one to take advantage of signal enhancement, noise reduction and high sample throughput. As a result, decreased detection limits and shorter analysis times can be obtained. The life of microbore columns depends on the degree of use and the procedures for clean-up of the loaded samples.

2.5. Electrochemical detectors

Amperometric and coulometric detectors have been reviewed by Kissinger [99–101]. Amperometric detectors are the most commonly used electrochemical detectors in HPLC. An amperometric detector usually consists of an

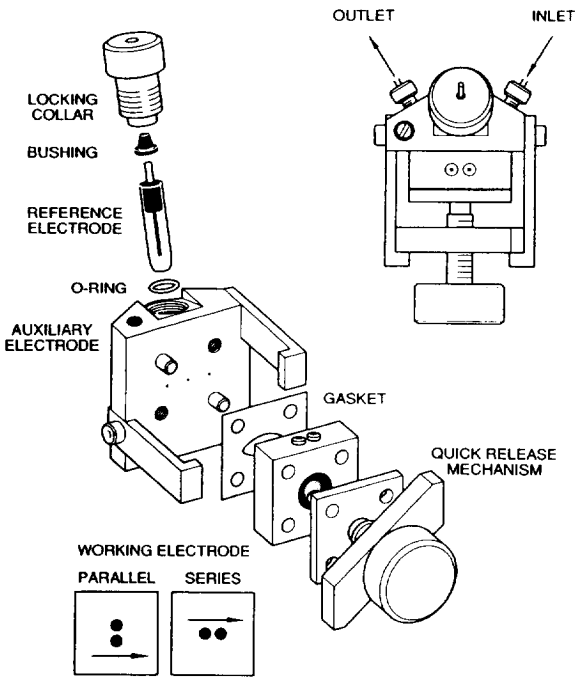

Fig. 4. Diagram of a thin-layer cell for electrochemical detection with HPLC. Dual electrodes can be arranged in parallel or series. (From the BAS-4C manual.)

electrode, which is part of a channel wall, formed by a fluorocarbon gasket (typically 50–80 μm) between two inert plastic blocks (Fig. 4) [101]. The dead volume of this type of detector is typically less than 1 μl. Under conventional HPLC conditions, these detectors convert 1–5% of the electroactive species present in solution [99]. Because of the small elution peak volume obtained from microbore columns, the electrochemical detector must be miniaturized to allow only a minimum extra-column contribution to peak broadening. The cell volume can be reduced to 0.15–0.25 μl by using a thinner gasket (12–16 μm). In general, a flow-cell volume of the order of 0.15–1 μl is required for columns of 1 mm I.D., and <0.1 μl is desirable for columns of <0.5 mm I.D. [102].

Coulometric detectors attempt to achieve 100% conversion of electroactive species of interest. The cell design allows a large active electrode surface area to be exposed to the solution, which leads to a higher background current and more noise. Coulometric detectors have been used successfully by several investigators [103–105]; however, the dead volume of coulometric detectors is typically larger than 2 μl. The performance of a microbore column in coulometric detectors can be strongly influenced by the extra-void volume broadening [92].

New electrochemical detectors have been developed for microbore HPLC–ED. The Antec Decade (acronym for digital electrochemical amperometric detector) (Analytical Technology, Leiden, Netherlands) contains a number of important features for maximizing the sensitivity and selectivity of microbore HPLC–ED. The Decade has been claimed to represent a new era in microbore and capillary HPLC–ED for ultratrace analyses [106]. The BAS UniJet (Bioanalytical Systems) (Fig. 5) is a new electrochemical detector with a miniaturized flow cell, providing a convenient platform for on-line electrochemical detection for microbore HPLC–ED [107]. A centrosymmetric radial flow thin-layer geometry is used with a solid reference electrode. Both of these new electrochemical detectors are capable of lowering the detection limit

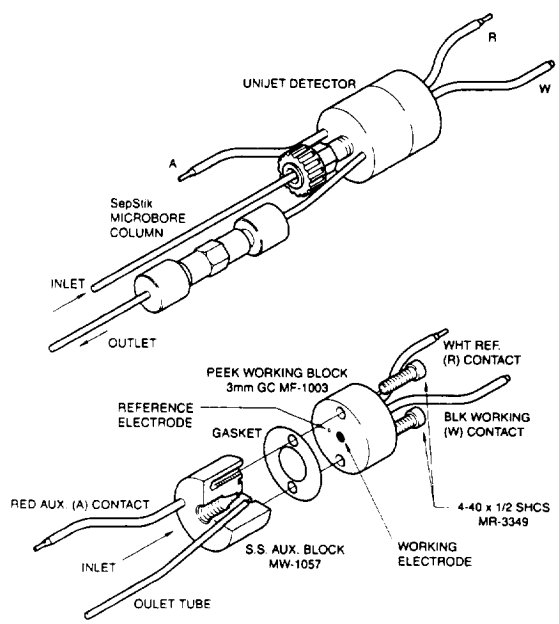

Fig. 5. UniJet amperometric detector cell for microbore HPLC. (From Ref. [107].)

of biogenic amines to 10^{-11} M (or <0.05 pg per injection).

2.6. Sample preparation procedures

For the determination of biogenic amines in tissues and biological fluids, a number of pretreatment protocols have been suggested and tried [108–114]. In order to achieve the best results, one has to consider the chemical nature of the analytes, the biological matrix and the possible presence of interfering compounds [115]. Furthermore, the selectivities of the HPLC–ED system should be considered in terms of procedures for clean-up of the samples. Single-step clean-up procedures are often used, but it should be borne in mind that two-step or multi-step clean-up procedures are often required. These clean-up methods require sample purification by means of ion exchange [108,109] or organic solvents [110,111], or extraction procedures with alumina [112,113]. Biogenic amine levels in biological fluids and tissue samples are diverse. As some are far below the detection limits of conventional HPLC systems, detection requires a large volume of the sample to allow the tedious pretreatment or preconcentration procedures. Recent developments with *in vivo* microdialysis and ultrafiltration procedures have produced clean samples, without further pretreatment, in very small volumes and with low concentrations of compounds of interest [116,117]. These clean samples are highly suitable for microbore column separation.

2.7. Temperature control

Temperature control in HPLC may be the most neglected factor. Many chromatographs are used with columns exposed to the air [101]. Therefore, electrochemical redox processes and electrode responses are adversely influenced by temperature changes in HPLC–ED. Microbore columns are most severely influenced by temperature changes. Changes of a few degrees affect a number of factors, such as diffusion coefficients, thermodynamics (retention time), redox kinetics (peak width) and background noise, which may significantly compromise quantification statistics. In fact, we have often observed these changes in our laboratories [116,117]. Therefore, both the microbore column and the detector cell should be thermally controlled. The BAS-200 (Bioanalytical Systems) and the Decade (Antec) come complete with an oven/Faraday cage for eliminating temperature fluctuations and external electrical interferences.

3. Microbore HPLC-ED analysis of biogenic amines

Microbore HPLC-ED has become very important for biomedical applications. High-speed separation is typical in microbore columns with conventional length (10–15 cm). These column sizes have recently been adopted for many biogenic amine assays [118,119]. Separations of biogenic amines can be accomplished in 1–2 min, or less, using more efficient microbore columns (14×1.0 mm I.D.) [120,121]. The driving forces for the rapid developments in microbore column HPLC–ED include very low detection limits, highly efficient separation power and lower solvent consumption. Applications of this technique for measuring biogenic amines in blood, tissues and cerebrospinal fluid are becoming increasingly popular.

3.1. Blood samples

In many laboratories, measurements of plasma serotonin, catecholamines and their metabolites have become routine. These samples contain thousands of compounds and proteins that are irrelevant to routine analysis. Therefore, large sample volumes for pretreatment and clean-up procedures are necessary to remove contaminants prior to HPLC assay. The amounts of biogenic amines and related compounds in blood are at trace levels in healthy subjects. In addition, blood sample volumes are frequently limited with experiments in small animals. Therefore, the analysis of biogenic amines must be performed close to the detection limit of a conventional HPLC–ED system. Plasma DA

and E have seldom been observed by means of HPLC–ED owing to the detection limits [17,122,123]. Microbore columns offer improved detectability for small sample amounts and trace concentrations. For instance, microbore HPLC–ED is suited to the analysis of plasma NE, E and DA, by a conventional alumina extraction method, at levels of a few pg/ml [124].

3.2. Tissue and cerebrospinal fluid

Evidence from neurochemical studies of post-mortem brain, measurements of biogenic amines from brain tissue and cerebrospinal fluid (CSF) have suggested that biogenic amines play etiological roles in many psychiatric and neurological disorders [1–4,14–17]. Biogenic amines in brain tissue and CSF have been analysed by conventional HPLC with a fluorimetric detection or ED [125,126]. However, measurements of some biogenic amines in CSF have met with inconsistent results. For instance, mean 5-HT levels in human lumbar CSF in healthy subjects have been reported to be in the range 0.05–12 ng/ml by HPLC–ED [127–130]. An inter-laboratory comparison study was carried out to determine mean 5-HT levels in lumbar CSF by microbore HPLC–ED [123]. The results showed that the mean concentration of CSF 5-HT should be less than 10 pg/ml. The mean 5-HT value and even the upper limit of this study were substantially lower than in all previous reports concerning normal human lumbar CSF [128–130]. Hence the extremely low concentrations of biogenic amines present in CSF may be accurately measured by microbore HPLC–ED. These results also shed light on the reinvestigation of the relevance of CSF and brain trace neurotransmitters to brain functions.

3.3. Microdialysates and ultrafiltrates

Microdialysis, as a new sampling technique, has come into widespread use for monitoring biogenic amines in various tissues and fluids, and also the release of peptides, drug levels for pharmacokinetics and a variety of chemical substances. It has become routine to isolate a few microliters of dialysate from a living animal using an *in vivo* microdialysis sampling technique [131–133]. Sample preconcentration is not practical with small volumes of dialysates. In addition, it is often necessary to determine amounts of individual compounds in the femtomolar range or below. Indeed, most publications from laboratories active in microdialysis suggest that microbore HPLC is the most general analytical method of choice. Over half of the microdialysis applications concern the analysis of biogenic amines by HPLC–ED. Many researchers wish to determine many analytes simultaneously within a dialysate. However, there is a need to analyze samples of very low concentration at which conventional columns (250 × 4.6 mm I.D.) have no place in most microdialysis applications. For example, the basal concentration of DA in rat striatum was estimated to be in the range 5–10 n*M*. Under normal conditions that included a 1–2-mm microdialysis probe with a 0.5–1 μl/min perfusion rate (the *in vivo* recovery was roughly estimated as 5–15%), and 5 μl of dialysate available for HPLC–ED, the results showed approximately 1.3–7.5 fmol of

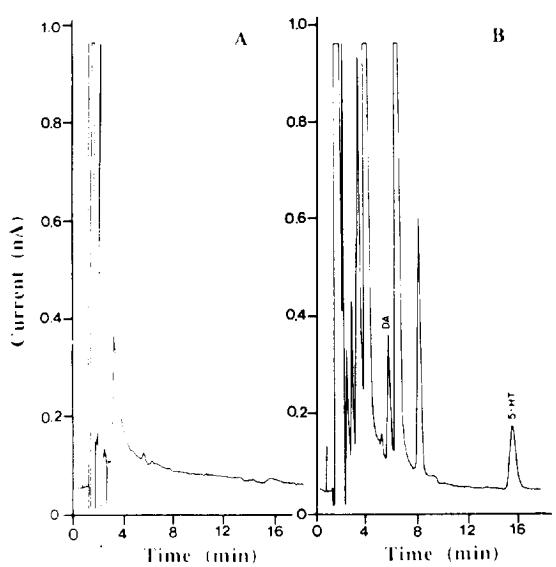

Fig. 6. Typical chromatogram of rat striatum dialysate containing DA and 5-HT. BAS microbore column; flow-rate, 70 μl/min; 5-μl sample loop. (A) Blank; (B) dialysate. (From Ref. [80].)

DA (Fig. 6) [60,62]. The basal levels of 5-HT in striatum or dosal vagal complex were also estimated to be 0.1–1.0 fmol per 5 μl of dialysate [60,62,134] (Fig. 7). Microbore column HPLC–ED was applied to investigate the above estimates with more than a 20-fold increase in the detection limit, when compared with conventional HPLC–ED [134].

Ultrafiltration (UF) techniques have been widely applied to plasma processing for drug monitoring assays [135,136]. The membrane role of the ultrafiltration is to act as a selective barrier, enriching certain compounds and depleting others [137]. This is very attractive for the isolation of small and hydrophilic molecules owing to the discrimination against high-molecular-mass substances, such as proteins and suspended matter, on the basis of size. Recently, UF probes were constructed and implanted in subcutaneous tissue to monitor glucose and catecholamines in living animals and human subjects [138,139]. Ultrafiltration techniques have also been applied to the measurements of biogenic amines and their metabolites in rat, cat and human plasma [116,117,140,141]. The collected dialysates or ultrafiltrate samples were free of protein and can be directly injected into the HPLC–ED system. Ultrafiltration techniques could be incorporated into a variety of pharmacological, physiological and behavioral experimental designs.

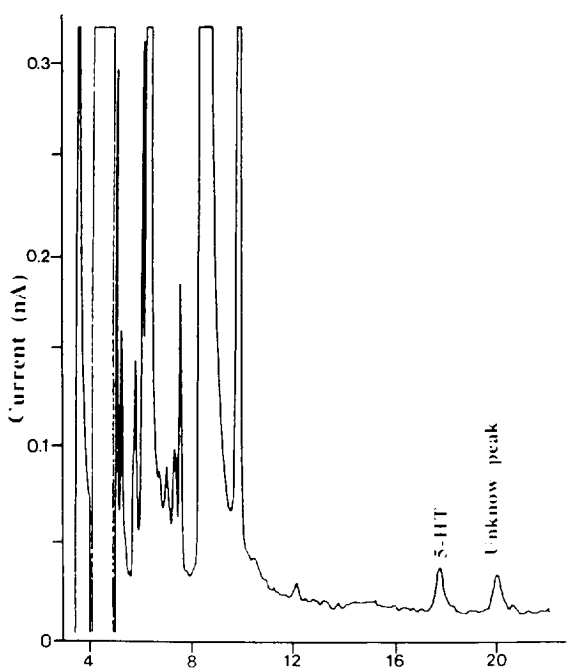

Fig. 7. Typical chromatogram of rat striatum dialysate collected 5 h after perfusion; 4.3 fmol of 5-HT were observed. BAS microbore column; flow-rate, 70 μl/min; 5-μl sample loop. (From Ref. [77].)

4. Conclusion

Since the first application of HPLC–ED, it has become an increasingly important analytical tool in neuroscience. Recently, microbore HPLC–ED has become the method of choice for the determination of trace biogenic amines and their metabolites. The ease of sample preparation, versatility of applications, sensitivity and ease of equipment maintenance make microbore HPLC–ED popular in neurochemical research. In early studies, problems with the specificity and sensitivity of the analytical procedures were responsible for the lack of agreement and accuracy in the results of different researchers. With the advantages of microbore HPLC–ED with regard to sensitivity and selectivity, these difficulties are now behind us. Henceforth, we may focus our aims on difficulties apparent in the multifactorial nature of disease states, and on persistent differences in experimental criteria employed by different groups. In addition, several factors, such as age, gender, diet and drug interactions, are known to affect the concentrations of biogenic amines and their metabolites in the body. Although very significant progress has been made recently, there is still room for improvement in microbore HPLC–ED. Simplified sample work-up procedures and more reliable microbore columns with sufficient separation power are two major areas where further work is needed. It is our belief that the microdialysis technique and ultrafiltration techniques coupled with the microbore HPLC–ED system may become essential for overcoming

analytical limitations in the determination of diverse concentrations of biogenic amines and related compounds.

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