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## Letter to the Editor

# Reduced variation in retention times of biogenic amines by temperature control in liquid chromatography with electrochemical detection

Sir,

It is known that in high-performance liquid chromatography (HPLC) with electrochemical detection (ED) large fluctuations in column temperature can affect retention times, peak heights and other separation characteristics. However, in air-conditioned or centrally heated laboratories temperature fluctuations are small during a 24-h period. The effects of these relatively small temperature fluctuations on separation characteristics have not been adequately investigated. The objectives of this study were (1) to investigate the magnitudes of changes in retention times of biogenic amines when separations are performed in an air-conditioned laboratory and (2) to determine if these changes can be reduced to acceptable levels by stabilizing the temperatures of column and mobile phase with a simple system of heating jackets and temperature controllers.

## EXPERIMENTAL

Separations of dopamine (DA), norepinephrine (NE), 3,4-dihydroxybenzylamine (DHBA), and serotonin (5-HT) were performed using an LC-4A amperometric detector, a Phase II, 5 $\mu$m, ODS, reversed-phase $C_{18}$ column, and a glassy carbon working electrode (Bioanalytical Systems, West Lafayette, IN, U.S.A.). The mobile phase (pH 3.0) included monochloroacetic acid (14.15 g/l), sodium hydroxide (4.675 g/l), EDTA (250 mg/l), octanesulfonic acid (300 mg/l), 1.4% tetrahydrofuran, and 2% acetonitrile. The flow-rate of the mobile phase was 1.7 ml/min, the sensitivity of the detector was 1 nA full scale, and the potential of the working electrode was 0.8 V with reference to a Ag/AgCl reference electrode.

The room, column and mobile phase temperatures were monitored with the help of temperature probes (Yellow Springs Instrument, Yellow Spring, OH, U.S.A.). In the first part of the study, separations were performed without controlling the temperature. In the second part, the temperatures of the column and mobile phase reservoir were maintained at 29.4–30.1°C and 43.2–44.0°C, respectively, with the help of custom-fitting heating jackets (Glas-Col, Terre Haute, IN, U.S.A.). The temperatures of the heating jackets on the column and mobile phase reservoir were controlled by Model 720 Lab Temperature Controllers (Dowty Electronics, Brandon, VT, U.S.A.).

## RESULTS AND DISCUSSION

During a 24-h period, the temperature in the air-conditioned laboratory fluctuated between 24.8 and 27.5°C (Fig. 1A). This fluctuation caused the column

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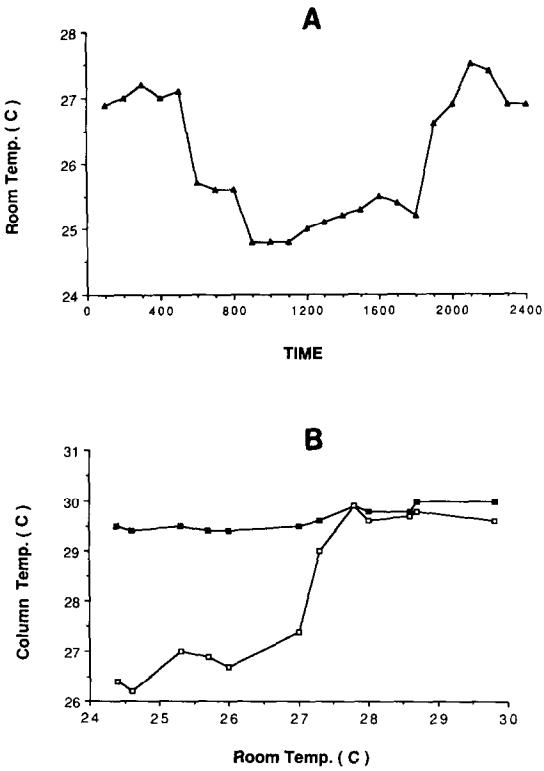


Fig. 1. (A) Fluctuations in room temperature in an air-conditioned laboratory during a 24-h period. (B) Fluctuations in the temperature of analytical column in an air-conditioned laboratory. Open squares = temperature of the column was not stabilized; closed squares = temperature of the column was stabilized at 29.4–30.1°C with the help of a heating jacket and a temperature controller.

temperature to vary between 26.2 and 29.9°C (Fig. 1B). The use of heating mantles on the column and mobile phase reservoir stabilized the column temperature between 29.4 and 30.1°C, although the room temperature continued to fluctutate as before.

When temperatures of the mobile phase and column were not controlled, the differences between the shortest and the longest retention times were 6.9%, 10.4%, 14.5% and 15.8% for NE, DHBA, DA and 5-HT, respectively (Table IA). By contrast, when temperatures of the column and mobile phase reservoir were stabilized the variability in retention times decreased to 0%, 4.1%, 3.8% and 5.1% for NE, DHBA, DA and 5-HT, respectively (Table IB).

These results indicate that variations in the retention times of biogenic amines can be reduced by stabilizing the temperatures of the column and mobile phase with a simple system of heating jackets and temperature controllers. When the column temperature was maintained within a range of 0.7°C, the longest retention times for DA, DHBA and 5-HT were only 5% or less longer than the shortest retention times, and the variation in the retention time of NE was undetectable. This represents a substantial narrowing of the variability in retention times and leads to increased confidence in the identity of the compounds being separated.

TABLE I

RETENTION TIMES OF BIOGENIC AMINES AND DHBA

A: Retention times were determined in an air-conditioned laboratory without stabilizing the temperatures of analytical column and mobile phase; the room and column temperatures fluctuated between 24.8 and 27.5°C and between 26.2 and 29.9°C, respectively. B: Retention times were determined in the same laboratory but the column and mobile phase temperatures were stabilized by a simple system of heating jackets; the room temperature fluctuated as before, but fluctuations in the column temperature were reduced to 0.7°C (between 29.4 and 30.1°C).

| *Trial* | *Retention time (min)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *A* | | | | *B* | | | |
| | *NE* | *DHBA* | *DA* | *5-HT* | *NE* | *DHBA* | *DA* | *5-HT* |
| 1 | 3.1 | 5.3 | 8.7 | 26.4 | 3.0 | 5.0 | 8.1 | 24.6 |
| 2 | 3.1 | 5.1 | 8.3 | 25.2 | 3.0 | 5.0 | 8.0 | 24.2 |
| 3 | 3.1 | 5.2 | 8.2 | 25.0 | 3.0 | 5.0 | 8.0 | 23.8 |
| 4 | 3.1 | 5.0 | 8.1 | 24.5 | 3.0 | 5.0 | 8.0 | 23.6 |
| 5 | 3.1 | 5.1 | 8.2 | 24.6 | 3.0 | 5.0 | 8.0 | 24.0 |
| 6 | 3.1 | 5.2 | 8.2 | 25.2 | 3.0 | 5.1 | 8.0 | 24.4 |
| 7 | 3.1 | 5.1 | 8.1 | 24.8 | 3.0 | 5.0 | 8.0 | 23.8 |
| 8 | 3.0 | 5.0 | 7.8 | 23.3 | 3.0 | 5.0 | 7.9 | 23.8 |
| 9 | 2.9 | 4.8 | 7.7 | 22.8 | 3.0 | 4.9 | 7.8 | 23.8 |
| 10 | 2.9 | 4.8 | 7.6 | 22.8 | 3.0 | 5.0 | 8.0 | 23.4 |
| 11 | 3.0 | 4.8 | 7.6 | 23.2 | 3.0 | 5.0 | 8.0 | 23.4 |
| 12 | 3.1 | 5.0 | 8.0 | 25.2 | 3.0 | 5.0 | 8.0 | 23.7 |
| Range | 0.2 | 0.5 | 1.1 | 3.6 | 0.0 | 0.2 | 0.3 | 1.2 |

The results indicate that temperature fluctuations during a 24-h period are rather substantial even in air-conditioned laboratories and lead to almost parallel changes in column temperatures and, consequently, to variations in retention times and annoying doubts and errors in identification of compounds. The temperature-controlling system used in this study minimizes these hazards by maintaining variability in the column temperature within a range of 0.7°C. Even this small variability can be eliminated by using a more sensitive temperature controller. On the whole, the temperature controlling system used in this study is simpler and less expensive than ovens, glass column jackets and cumbersome circulating water baths.

Another advantage of the system is that it permits preheating of the mobile phase just before its entry into the column. This is considered essential for achieving maximum efficiency in HPLC–ED, since it prevents formation of a temperature gradient in the column and the consequent peak-splitting phenomenon[1,2].

It has been shown that high column temperatures reduce retention times by as much as 40% and increase resolution by producing sharper and higher peaks[3]. The temperature controlling system used in this study has the capacity to maintain column temperature at higher levels of 50 or 60°C. The resultant increase in resolution and decrease in run times would be especially useful when a large number of samples is being analyzed[3]. Excessive increases in column temperature, however, are not advisable when the compounds being separated are thermally labile and when low boiling solvents are used in the mobile phase.

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