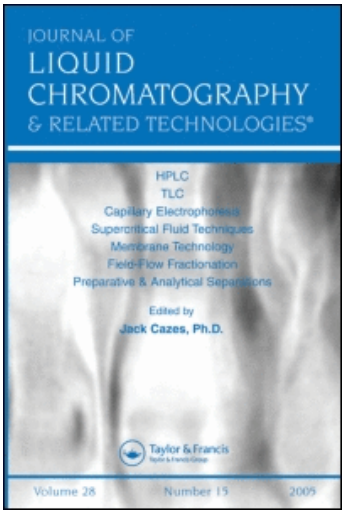

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Role of the Dissolution Mechanism in Determining Chromatographic Separation of Biogenic Amines from Their Precursors and Metabolites by HPLC-EC

Q. Meng[a]; Y. F. Chen[a]; S. Oparil[a]

[a] Hypertension Research Program Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama

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ROLE OF THE DISSOLUTION MECHANISM IN DETERMINING CHROMATOGRAPHIC SEPARATION OF BIOGENIC AMINES FROM THEIR PRECURSORS AND METABOLITES BY HPLC-EC

Q. Meng, Y. F. Chen, and S. Oparil
Hypertension Research Program
Department of Medicine
University of Alabama at Birmingham
Birmingham, Alabama 35294

ABSTRACT

The current study was designed to elucidate the theoretical basis for chromatographic separation of biogenic amines on an octadecyl-silica (C-18) reverse phase column by determining the intermolecular forces between the solute and the stationary phase. The solutes mass transfer diffusion and the heat effect between solutes and stationary phase were assessed by a convenient method. This study demonstrates that the dissolution mechanism plays a major role in the process of chromatographic resolution of biogenic amines and their precursors and metabolites by HPLC-EC.

INTRODUCTION

The concurrent quantitative determination of biogenic amines and their precursors and metabolites using LCEC is currently the most frequently employed method of assessing the activities of specific monoamine containing neuronal populations in brain (1).

In the last several years, a number of procedures for separating biogenic amines, their precursors and metabolites using the octadecyl-silica (C-18) reverse phase analytical column have been described (2). The influence of the composition of the LC mobile phase on the retention time and chromatographic characteristics of these compounds has been studied extensively (2). This paper assesses the role of the interaction between the solutes and the stationary phase in the chromatographic process. Based on the retention behavior and band broadening of biogenic amines and their metabolites on a reverse phase column, the effects of the solute mass transfer diffusion in the stationary phase on the separation process were assessed. These data clarify the mechanisms by which biogenic amines and their precursors and metabolites are separated by HPLC-EC and provide useful information for selecting the mobile phase composition necessary for optimizing chromatographic resolution.

Experimental

In our laboratory, 4-hydroxy-3-methoxyphenylglycol (MOPEG), norepinephrine (NE), epinephrine (EPI), dopamine (DA), 3-4 dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid (5HIAA), homovanillic acid (HVA) and 5-hydroxytryptamine (5HT) contents of standards and tissue samples are routinely determined concurrently using HPLC-EC. The HPLC system utilizes a Waters M-45 solvent delivery system (Waters Associates, Milford, MA), a Waters WISP 710 automatic injector and Altex ultrasphere (Beckman Instruments, Columbia, MD) ODS 5μm reverse phase column with a BAS LC-23A (Bioanalytical Systems, Inc) column heater. The potential of the BAS TL-5A glassy carbon working electrode versus a Ag/AgCl reference electrode is set a +0.75 V. The BAS LC-4A amperometric detector is set at 2 nA/V sensitivity. Chromatographs are recorded on a Hewlett-Packard HP-3390A integrator (Hewlett-Packard Company, Avondale, PA). The mobile phase consists of 0.02M citric acid (650

ml); 0.02M sodium dihydrogen phosphate buffer containing 0.269mM EDTA (300 ml); 0.008% (W/V) sodium octyl sulphate (SOS); 5% (V/V) acetonitrile, and 0.23% (V/V) phosphoric acid and is adjusted to pH 4.0 with 10N sodium hydroxide.

On the basis of a plot of height equivalent to a theoretical plate (H) vs. flow rate (u), the diffusion coefficients (D_s) of solute in the stationary phase have been determined. From a plot of the logarithem of capacity factor K' versus the reciprocal of absolute temperature 1/T, the heat effect (ΔH) between stationary phase and solutes has been evaluated.

RESULTS AND DISCUSSION

In HPLC, band broadening effects can be expressed as follows (3):

$$H = B/U + CsU + (1/2\lambda dp + Dm/dp^2\ U)^{-1} \quad (I)$$

where H is the height equivalent to a theoretical plate; B represents the longitudinal diffusion; Cs represents mass transfer resistance caused by solute diffusion in the stationary phase; λ is the tortuosity of the packing material, which is approximately equal to 1, as the microspheres are porous; dp is the diameter of the microspheres in the stationary phase; U is the actual mobile phase velocity, and Dm is the diffusion coefficient of the solute in the mobile phase. Therefore, the term $(1/2\ \lambda dp + Dm/dp^2\ U)^{-1}$ represents a coupling of the eddy diffusion and mobile phase terms. It is well known that the diffusion coefficient of a small solute molecule (Dm) in water is approximately equal to $10^{-5}cm^2/sec$, and usually the flow rate of mobile phase (U) used is of the order of mm/sec, and the particle size of the stationary phase (dp) is 5 μm, so the contribution of the last term is mainly due to the $2\lambda dp$. Since the diffusion coefficient of solute is about 10^5 times smaller in the liquid phase than in the gas phase, this longitudinal term (B/U) plays no practical role in band broadening in LC. Thus the equation describing band broadening effects can be simplified as follows:

$$H = CsU + 2\lambda dp. \qquad (II)$$

In general, Cs is a function of the diffusion coefficient (Ds) of the solute in the stationary phase, the relative migration rate of the solute in the mobile phase, the particle size (dp) and its pore structure parameters. Cs can be computed from Dawkins equation (4).

$$Cs = R(1-R)\ dp^2/30Ds \qquad (III)$$

where R is the ratio of non-retention volume V_0 and the elution volume of the sample V_R. Thus Ds values of solutes can be obtained from the H-U curves and Eqs (II, III). The dynamic distribution behavior of solute diffusion in the stationary phase plays a central role in the chromatographic process which describes the solute mass transfer in the stationary phase and equilibrium distribution during sample separation. Generally, the solute diffusion coefficient in the mobile phase (Dm) depends upon the molecular weight of the solvent, temperature, solvent viscosity and the molar volume of the sample molecule. A larger solute diffusion rate leads to a faster equilibrium distribution of solute between stationary and mobile phases. Separation then results from different velocities of migration as a consequence of differences in equilibrium distribution. Table 1 shows the solute diffusion coefficients (Ds) in the stationary phase of the reverse phase column and the relative retention behavior (R) of biogenic amines and their metabolites. Thus, the solute diffusion coefficients and the retention times of these compounds are closely correlated.

According to $\Delta(\Delta G^\circ)=RT\ln\alpha$

Where $\Delta(\Delta G^\circ)$ is the difference in free energies of distribution for two components, α is the net retention time for the two components. When $\alpha=1$, the resolution is zero, regardless of the number of theoretical plates in the dynamic process, since there must be some difference in the themodynamic distribution behavior of two components for separation to occur. Relative retention relates to differences in interaction of the two components in the mobile

TABLE 1
Diffusion Coefficients (Ds) and Relative Retention Times (R) of Biogenic Amines and Their Metabolites

	$Ds(X10^{-5}\ cm^2/sec)$	R (Vo/VR)
MOPEG	6.0	0.38
NE	5.7	0.32
EPI	5.4	0.25
DOPAC	4.3	0.20
DA	4.1	0.15
5HIAA	3.6	0.13
HVA	3.3	0.08
5HT	3.0	0.05

Chromatographic conditions same as experimental.

and stationary phases accordingly to $\ln K = -\Delta H/RT + \Delta S/R$, where ΔH is the free enthalpy which relates to solute migration from stationary phase into mobile phase. The themodynamic ΔH value describes the interaction behavior between solute and stationary phase exactly. The heat effect ΔH was calculated from a plot of logarithm of the capacity factor K versus the reciprocal of absolute temperature 1/T as listed in Table 2.

The results show that the larger the heat effect ΔH, and the stronger the intermolecular forces between solutes and stationary phase, the longer the retention time. It is well known that the retention of compounds on C-18 reverse phase columns is dependent on both the hydrophobic interactions between the solute and nonpolar stationary phase and the hydrophilic properties of the mobile phase. Our data demonstrate that the themodynamic function of intermolecular forces affects the solute mass transfer in the stationary phase which, in turn, causes the equilibrium distribution of solute between mobile and stationary phases during the chromatographic process. Therefore, in a fundamental sense, the kinds and magnitudes of intermolecular forces are responsible for determining the ability of an LC system to effect a separation.

Solute mass transfer into the stationary phase in LC is dependent on the dissolution mechanism in the chromatographic

TABLE 2

Capacity Factor (K) and Heat Effect (ΔH) of Biogenic Amines and Their Metabolites

$T(X10^{-3})$		MOPEG	NE	EPI	DOPAC	DA	5HIAA	HVA	5HT
3.19	lnK	1.08	1.54	2.37	2.83	3.71	4.24	5.18	6.06
3.30	lnK	1.17	1.73	2.60	3.13	4.05	4.65	5.60	6.59
3.41	lnK	1.27	1.93	2.86	3.39	4.38	5.02	5.99	7.02
ΔH		0.73	1.55	1.91	2.18	2.64	3.09	3.16	3.81

Unit of ΔH: Kcal/g-mol, K= (t-to)/to
Chromatographic conditions same as experimental.

process. From our experiment, the calculated heat effect (ΔH) is 2-3 Kcal/g-mol, as in Table 2. This value is similar to the heat of solution but not to that of adsorption. Therefore, the dissolution mechanism is an important determinant of the chromatographic separation of biogenic amines and their metabolites by LC and should be taken into consideration when selecting conditions necessary for optimizing chromatographic resolution of these compounds.

Taken together, results of the current study demonstrate that the intermolecular forces between biogenic amines and the stationary phase partially account for the retention time of these compounds on C-18 reverse phase columns. Further, the current results provide evidence that the dissolution mechanism plays an important role in determining the chromatographic separation of biogenic amines from their metabolites by HPLC-EC. Consideration of the intermolecular forces between the solute and the stationary phase should facilitate the selection of conditions which will enhance chromatographic resolution of these compounds on C-18 reverse phase columns.

REFERENCES

1. Chen, Y. F., Jin H., Gist R., and Oparil S., Blunted responsiveness of posterior hypothalamic norepinephrine to quinpirole in DOCA/NaCl hypertensive rats. Brain Res. Bull. (in press), 1987.

2. Mayer, G. and Shoup, R., Simultaneous multiple electrode liquid chromatographic-electrical assay for catecholamines, indole-amines and metabolites in brain tissue. J. Chromatogr., 225, 533, 1983.

3. Kirkland, J. J., Modern Practice of Liquid Chromatography, John Wiley and Sons, Inc., New York, NY, 1971.

4. Dawkins, J. V. and Yeadon, G., High-performance gel permeation chromatography with silica macrospheres: mass-transfer dispersion and poly-dispersity of polystyrene, J. Chromatogr., 206, 215, 1981.