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Temperature of the eluent: a neglected tool in high-performance liquid chromatography?

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Abstract

The influence of eluent temperature on efficiency in high-performance liquid chromatography with special reference to the role of viscous heat dissipation is investigated for water-jacket and air-bath thermostatted columns. When separations are performed on well-thermostatted large-diameter columns packed with small particles or on analytical columns operated at high flow-rates, the additional thermostatting of the eluent to a specific temperature below the column temperature turns out to have some advantages. The precooled eluent causes the development of a temperature profile in a radial direction which is opposed to and compensates the radial temperature profile in the column caused by viscous heat dissipation. For columns that are thermostatted in an air bath, a similar effect is observed, but the strong dependence of the column temperature on the eluent temperature results in a poor reproducibility if the eluent is not thermostatted itself.

Keywords: Eluent temperature; Temperature effects; Column efficiency; Viscous heat dissipation

1. Introduction

In HPLC there has always been a tendency to underestimate the influence of temperature on separation. Even today columns are often used without thermostatting, as a mere appendix of the apparatus or the pump. However, since temperature influences both the thermodynamics and kinetics of the separation process, controlling the column temperature should not be neglected. The different effects of temperature on retention factor, viscosity, diffusivity and plate height are well investigated [1–16] and have been applied extensively to optimize HPLC separations.

However, the temperature inside an HPLC column is not uniform: the flow of the viscous mobile phase through the packing bed generates heat at a constant rate over the whole length of the column. Depending on the way the column is thermostatted and the characteristics of the mobile phase and the packing material (particularly heat capacity and heat conductivity), longitudinal and radial temperature gradients within the column may be formed by this viscous heating. Its appearance and consequences for column performance have already been discussed by Halász et al. [17] and Horváth and Lin [18]. Later, the phenomenon of viscous heat dissipation was

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extensively treated in theory and investigated experimentally by Poppe and co-workers [19,20].

They derived (heat balance) equations which help to predict the current, actual temperature at different positions within the column for well-thermostatted columns (e.g. using a water jacket). The possible effects of viscous heat dissipation on temperature profile, flow-velocity profile and zone broadening in such a column are illustrated in Fig. 1A for the case in which the eluent enters the column with a temperature equal to the column wall temperature. The viscous heat generated leads to a temperature rise in the centre of the column. Due to the relatively low heat conductivities of the mobile phase and the packing bed, a parabolically shaped, radial temperature gradient is formed. At a certain longitudinal distance from the inlet, the temperature difference between the centre and the wall (the temperature profile) remains constant because the amounts of excess heat and conducted (dissipated) heat are equal. This distance, called the relaxation length, is proportional to velocity, heat capacity, and square of the column radius, and inversely proportional to heat conductivity in the radial direction, while the maximum temperature rise along the column axis is proportional to velocity, square of the column radius and pressure drop over units of the column. It is also inversely proportional to heat conductivity in the radial direction at this length [19]. Because of the radial temperature gradient, a flow profile with the highest flow velocity in the centre of the column is formed. These different flow velocities give rise to multi-path zone broadening in addition to the zone broadening which can be described by the usual plate height equations.

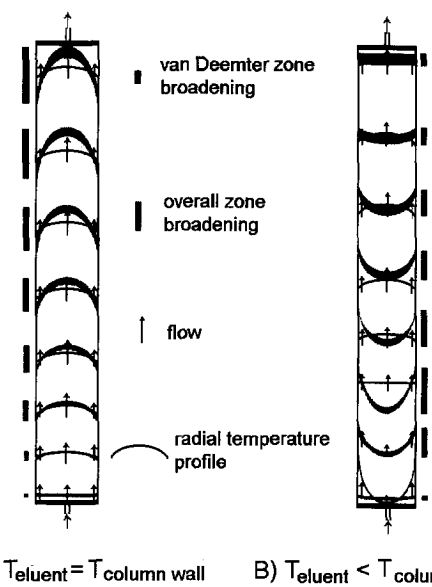

Fig. 1. Influence of eluent temperature on thermal conditions, flow and band broadening in a well-thermostatted column: (A) eluent temperature equal to column wall temperature; (B) eluent temperature below column wall temperature.

If, however, the mobile phase enters the column with a temperature lower than that of the column wall (illustrated in Fig. 1B), an opposite radial temperature gradient is formed in the first part of the column. This leads to lower flow velocities in the column axis at the inlet of the column. Thus, it may compensate for the positive gradient in the second part arising from the viscous heat. At a certain optimum temperature difference between column and mobile phase, the different flow velocities are just offset, and hence no additional zone broadening results at the column outlet.

On the basis of these considerations, we studied the influence of temperature differences between eluent and column wall on efficiency for both analytical and semi-preparative columns packed with 5-μm particles.

2. Experimental

2.1. Set-up A for analytical columns

A HPLC pump Model 501 (Waters, Milford, MA, USA) and an UV detector type ERC-7210 (Erma Optical Works, Tokyo, Japan) working at 254 nm were used. Pressure was controlled by a flow-through pressure gauge (Model CFP-224; Span Instruments, Plano, TX, USA). Chromatograms were recorded with a digital integrator Model 2500 (Merck-Hitachi, Darmstadt, Germany). An injection valve Model 7125, with a 20-μl sample loop (Rheodyne, Cotati, CA, USA)

and a coil made of 540 cm stainless-steel tube (0.5 mm I.D.) were placed in a Peltier thermostat (courteously manufactured to our specifications by W.O. Electronics, Langenzersdorf, Austria). The construction allowed adjustment of the temperature of the mobile phase from 4°C to 60°C up to a flow-rate of 7 ml/min. Columns were thermostatted by a water-jacket system (custom-made). Water was circulated by means of a liquid thermostat type B (Lauda, Lauda-Königshofen, Germany). Investigations in circulated air were done with an air thermostat Model CO 200 (Jasco, Groß-Zimmern, Germany). PEEK tubing (0.25 mm I.D.) used to connect the injection valve and the columns was as short as possible (18 cm).

2.2. Set-up B for preparative columns

A liquid chromatograph HPLC gradient system Series 800 (Biorad, Richmond, CA, USA) with two pumps (Model 1350), a UV–Vis detector with variable wavelength (Model 1790), a PC integrator (Model 804) and an injection valve (Model 7125, Rheodyne) with sample loops of 20, 200 and 2000 μl were used. Thermostatting of the eluent was accomplished by immersion of 70-cm coiled stainless-steel tubing (0.8 mm I.D.) and the injection valve into a water reservoir of a liquid thermostat type B (Lauda). Columns were thermostatted using a water-jacket system as above.

Temperatures were controlled by a mercury thermometer (Lauda), a thermocouple thermotester Model PT 2282 A (Philips, Hamburg, Germany) and a custom-made Ni–Cr–Ni thermocouple.

2.3. Columns

A commercial stainless-steel cartridge 250 × 4 mm I.D., 5 μm, LiChrosphere 100 RP-8 end-capped column, porosity 0.742 (Merck, Darmstadt, Germany), was used for investigations in the reversed-phase mode.

The influence of the eluent temperature in the normal-phase mode was investigated on a commercial stainless-steel cartridge 250 × 4 mm I.D., 5 μm, LiChrosphere 100-NH_2 column, porosity 0.824 (Merck).

For preparative separations a 5-μm C_{18}-Si 100 stainless-steel column 250 × 22 mm I.D., porosity 0.734 (Serva, Heidelberg, Germany), was used.

2.4. Chemicals

All solvents and solutes were of analytical grade.

2.5. Procedures

Peak widths were measured at half the maximum heights. From these values plate numbers and resolution were calculated. Resolution of peaks not separated to half of the heights was calculated as the quotient of the valley depth and the mean of the heights of both peaks (valley separation).

Systems were allowed to reach thermal equilibrium before measurements were done (usually 10 min were sufficient).

3. Results and discussion

The experiments were performed on different HPLC systems which allowed independent thermostatting of eluent and column. The impressive improvement of resolution that can be achieved by decreasing the eluent temperature ($T_{e,adjusted}$) relative to the column temperature is illustrated in Fig. 2. More detailed results indicating the dependence of plate numbers and resolution on eluent temperature for the RP-8 column are shown in Fig. 3. It can be seen that the influence of eluent temperature is small when a flow-rate of 1 ml/min is applied, which corresponds to a pressure drop of 97 bar. Yet there is a weakly pronounced maximum at an eluent temperature that is lower than the column wall temperature. At a flow-rate of 3 ml/min, the inlet pressure increases to 300 bar. At identical temperatures for eluent and column (30°C), there is obviously a considerable contribution to overall zone broadening by heat dissipation because the plate number drops to 3000. However, cooling of the

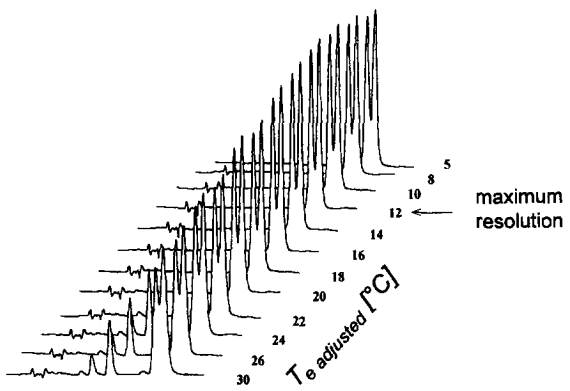

Fig. 2. Influence of eluent temperature on separation of benzene, naphthalene, phenanthrene and anthracene on the RP-8 column; mobile phase, acetonitrile–water (66:34); flow-rate, 3 ml/min; inlet pressure, 300 bar.

mobile phase down to 12°C at a constant column temperature of 30°C doubles the plate number again to 6000 and improves the resolution of the peak pair phenanthrene–anthracene from 0.65 to 1.11.

Primarily viscous heat is a function of the pressure drop over the column. The dissipation of this heat also depends on the heat capacity and heat conductivity of the mobile phase [17,19]. Thus, despite the low pressure drops, the influence of the eluent temperature is also observed in normal-phase HPLC. The advantage of eluent thermostatting for a separation of nitrotoluenes using an aminopropyl silica column (NH_2 column) and heptane–methyl-*tert.*-butyl ether (80:20) as eluent is presented in Fig. 4.

An optimum resolution can always be observed at an eluent temperature lower than the column wall temperature independent of the chosen column wall temperature. The difference between the optimum eluent temperature and the column wall temperature increases with decreasing column wall temperature, caused by the

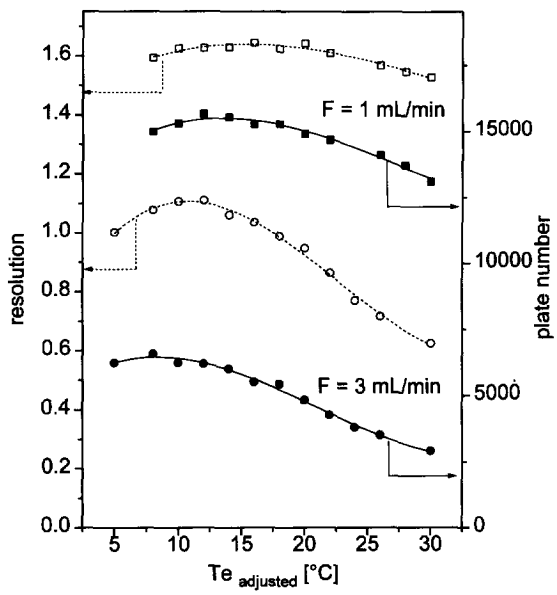

Fig. 3. Dependence of plate numbers and resolution on eluent temperature for the RP-8 column. Column thermostatted at 30°C; mobile phase, acetonitrile–water (66:34); flow-rates (F), 1 ml/min (inlet pressure 97 bar) and 3 ml/min (inlet pressure 300 bar). Dashed line: resolution of phenanthrene–anthracene. Solid line: plate numbers for naphthalene.

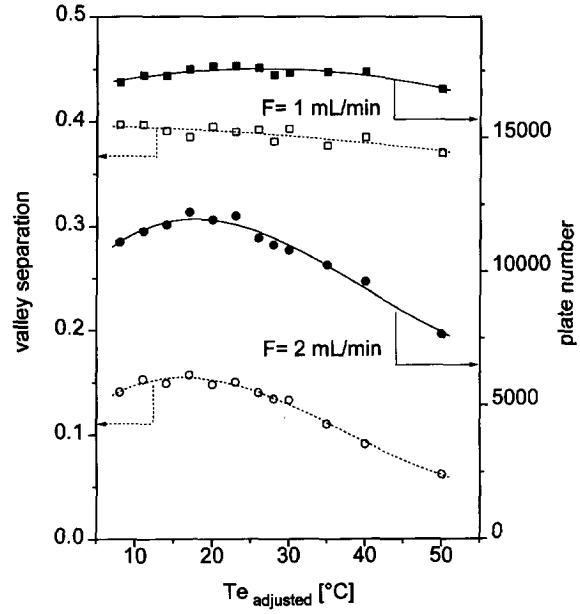

Fig. 4. Dependence of plate numbers and separation on eluent temperature for the NH_2 column. Column thermostatted at 30°C; mobile phase, heptane–methyl-*tert.*-butyl ether (80:20); flow-rates (F): 1 ml/min (inlet pressure 43 bar) and 2 ml/min (inlet pressure 94 bar). Dashed line: valley separation of 3-nitrotoluene/4-nitrotoluene. Solid line: plate number for 2,6-dinitrotoluene.

increasing viscosity of the mobile phase and the resulting increasing pressure drops.

Taking into consideration that the maximum axial temperature rise calculated according to equations derived by Poppe et al. [19] is 1.72°C (under the conditions described in Fig. 3) and the calculated relaxation length is 22 mm, the observed optimum eluent precooling of 18°C appears to be far too large. Furthermore, measurements of the temperature on the column outlet (same conditions as in Fig. 3) showed a mean eluent temperature only 0.5°C higher than the column wall temperature. Therefore, it was necessary to investigate more closely whether the eluent entered the packing bed with the assumed adjusted temperature.

To check the true temperature of the eluent at the entrance into the packing bed, we monitored it by positioning a thermocouple behind the inlet frit. The results using acetonitrile–water (66:34) as eluent are shown in Fig. 5. The eluent at the entrance of the packing bed has a temperature substantially different from that in the eluent thermostat. This is caused by the thermal contact of the eluent with the column head and the inlet frit. The degree of warming up depends on the flow-rate and whether the frit has a direct contact with the column wall. According to this graph the packing bed entrance temperature of the acetonitrile–water (66:34) mobile phase at a flow-rate of 3 ml/min and thermostatted to, for example, 12°C is 22°C. This optimum precooling of 8°C below the column temperature is within the expected range. For the heptane-methyl-*tert.*-butyl ether (80:20) mobile phase, a similar effect is observed.

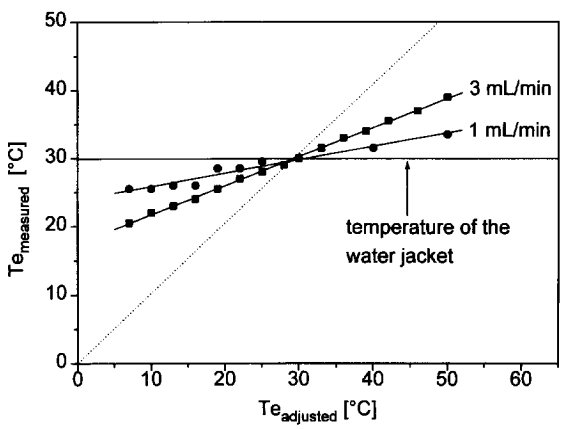

Fig. 5. Difference between adjusted eluent temperature and the measured temperature of the eluent at the entrance of the packing bed. Column thermostat: water jacket, 30°C; mobile phase, acetonitrile–water (66:34). Dotted line: $T_{e,expected}$ versus $T_{e,adjusted}$. Solid lines: resulting temperature gradient at the entrance of the eluent into the packing bed; flow-rates, 1 ml/min and 3 ml/min.

Assuming that the eluent will suffer similar changes in temperature due to its contact with the outlet frit and the column end as it does at the inlet side, the actual eluent temperature at the end of the packing bed just before the frit must be even higher than measured. With the aid of the slopes of the curves in Fig. 5, the actual difference between the mean temperature of the eluent and the column wall temperature at the end of the packing bed can be calculated as 1.2°C, which agrees well with the theoretically calculated value of 1.72°C for the maximum axial temperature rise under the chosen conditions (3 ml/min, RP-8 column).

All of the above results have been obtained using a well-thermostatted column (i.e. using a water bath thermostat). A less efficient means of thermostatting (an air-bath system) gives similar results for the RP-8 column concerning the effects of eluent precooling on plate numbers and resolution: similar maximum curves can be observed (see Fig. 6). However, there is a significant influence of the eluent temperature on retention data for a column thermostatted in circulated air (Fig. 7A) because the eluent temperature strongly affects the column temperature in such a system [20–22]. Already at a flow-rate of 1 ml/min, retention significantly depends on eluent temperature, while the changes in retention data at higher flow-rates are no longer acceptable. This is not the case when the column is thermostatted with a water bath (Fig. 7B). Therefore, an air-bath thermostat is not recommended if reproducible, constant retention data are to be obtained.

The influence of viscous heat dissipation is particularly pronounced when large-diameter columns packed with small particles are used.

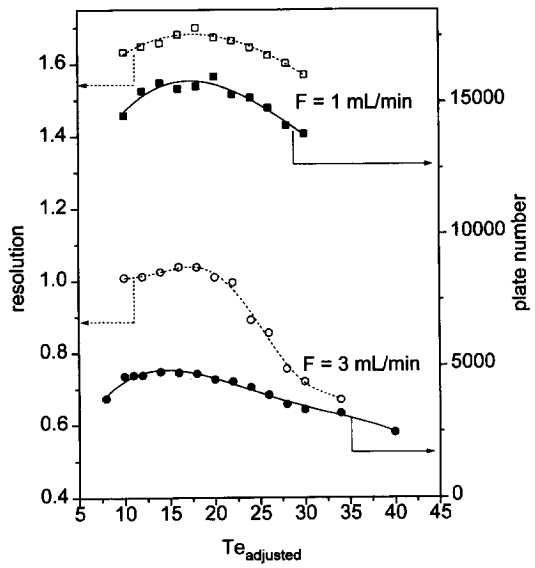

Fig. 6. Dependence of plate numbers and resolution on eluent temperature for an air-bath thermostatted RP-8 column. Column thermostatted at 30°C; mobile phase, acetonitrile–water (66:34); flow-rates, 1 ml/min and 3 ml/min. Dashed lines: resolution of phenanthrene/anthracene. Solid lines: plate numbers for naphthalene.

For the preparative separation of casomorphin-peptides on a 250 × 22 mm I.D. column packed with a 5-μm RP-18 material, a column temperature of 50°C has to be chosen. Most probably this temperature is necessary because of the small diffusion coefficients of the peptides. A further improvement of resolution could be achieved by decreasing the eluent temperature. The optimum resolution was found at an eluent temperature of 36°C. When the eluent temperature was reduced even further, resolution deteriorated again.

Optimizing the eluent temperature not only enables sufficient resolution but also renders it possible to increase the injection volume. In Fig. 8 maximizing the sample throughput for a preparative separation of a tetrapeptide-4-anilide from a synthesis mixture is illustrated. At precooling the eluent 20°C below the column wall temperature (25°C), an increase of injection volume to even 2 ml is achieved with still satisfactory separation.

4. Conclusions

In most conventional HPLC applications, the influence of the eluent temperature is not taken into account. Our investigations on water-jacket thermostatted HPLC columns showed that the influence of viscous heat dissipation on efficiency of analytical columns operating at high flow-rates and on preparative separations on large-diameter columns packed with small particles is not negli-

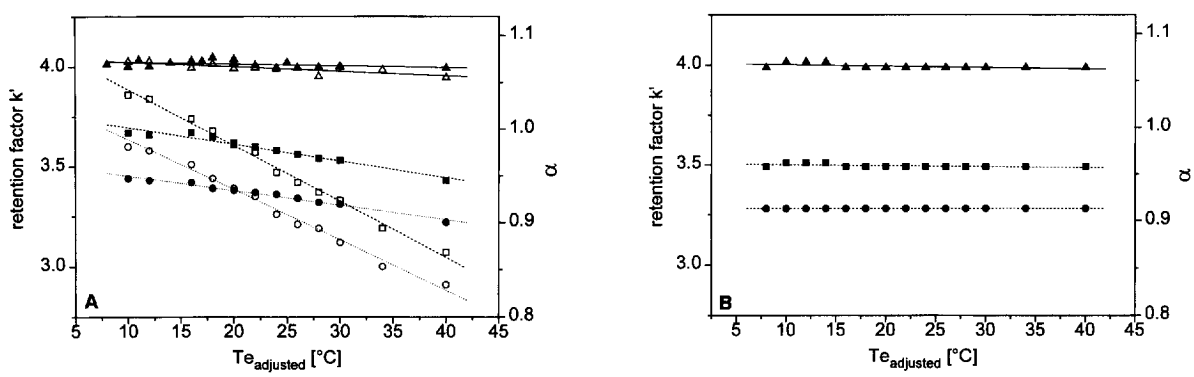

Fig. 7. Dependence of retention factors and selectivity on eluent temperatures. Column: RP-8 column thermostated at 30°C. Mobile phase, acetonitrile–water (66:34). Broken lines: retention factor k' for anthracene, 1 ml/min (■); phenanthrene, 1 ml/min (●); anthracene, 3 ml/min (□); phenanthrene, 3 ml/min (○). Solid lines: α of anthracene/phenanthrene, 1 ml/min (▲); α of anthracene/phenanthrene, 3 ml/min (△). Hold-up time determined with thiourea. (A) Column thermostatted in circulated air. (B) Column thermostatted by a water jacket (identical k' and α values were measured for flow-rates of 1 ml/min and 3 ml/min).

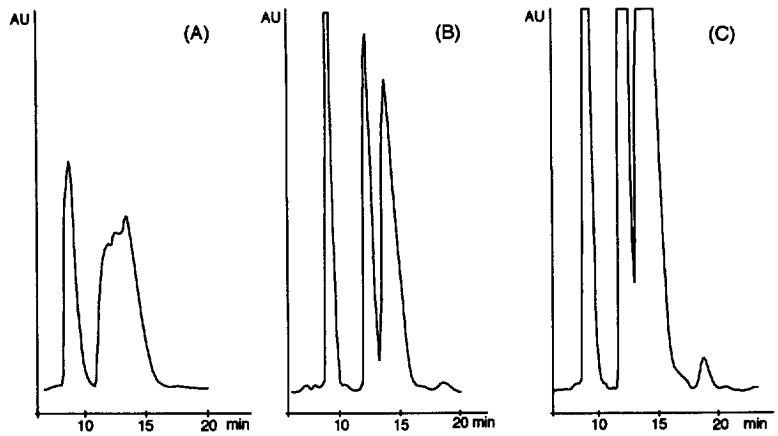

Fig. 8. Improvement of sample throughput by optimum thermal conditions for a preparative separation of a tetrapeptide-4-anilide (last peak) from the synthesis mixture. Column: 250 × 22 mm I.D., C_{18}, 5 μm; mobile phase, acetonitrile–0.02 *M* phosphate buffer pH 3.5 (22:78); detection wavelength, 280 nm; flow-rate, 8 ml/min. (A) Eluent thermostat temperature (T_e):column water-jacket temperature (T_w) = 25°C:25°C; sample volume, 200 μl (c = 0.75 mg/ml). (B) T_e:T_w = 5°C:25°C; sample volume 200 μl (c = 0.75 mg/ml). (C) T_e:T_w = 5°C:25°C; sample volume 2 ml (c = 5 mg/ml).

gible. Thermostatting of the eluent at a specific temperature below the column temperature improved resolution of critical peak pairs on a normal-phase and a reversed-phase column. Likewise, the potential of a preparative HPLC system could be utilized more effectively by controlled precooling of the eluent.

It could be shown that the optimum difference between the column wall temperature and the temperature of the eluent thermostat is not dependent solely on separation conditions (such as eluent composition, dimensions of the column and packing material, and column temperature). Also, the influence of the construction of the column head and arrangement of the HPLC system has proven to be of similar importance for the precooling of the eluent.

Finally, the effect of eluent temperature on efficiency and retention data of an analytical reversed-phase column thermostatted in an air bath was investigated. Precooling of the eluent gives results similar to those found for the water-bath system. However, due to the strong dependence of the retention factor on the eluent temperature, independent thermostatting of the eluent is highly recommended if reproducibility is desired.

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