CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

# Dial-A-Mix Mobile Phases

**Purpose**
To compare several methods of mixing mobile phases on the BAS 200 Chromatograph.

The BAS 200 Chromatograph has a ternary gradient solvent delivery system, which allows the on-line mixing of solvents from 3 bottles. One might assume that a totally aqueous buffer in bottle A could be mixed on-line with neat organic solvents from bottles B and C to create a gradient. An obvious extension of this technique would be the on-line mixing of A and B to create an isocratic mobile phase (Dial- a-Mix). However, such on-line mixing of totally aqueous and neat organic solvents can create chromatographic problems.

As anyone who has mixed two different solvents knows, the physical and chemical properties of the mixture are not necessarily the sum of their individual properties. Both permanent (viscosity) and temporary (temperature) changes may occur. For example, acetonitrile/water mixtures absorb heat (endothermic) while methanol/water mixtures produce heat (exothermic). With methanol/water mixtures in particular, heat production will lead to outgassing (because gas solubility decreases with temperature). Dial-a-Mix techniques can thus result in the release of gas bubbles, causing erratic pressure fluctuations and poor chromatographic performance.

The best method to avoid the problem of outgassing is to mix your isocratic mobile phase, including the organic solvent, at the lab bench, and then remove bubbles and dissolved gases. Removal of dissolved atmospheric gases can be accomplished by vacuum filtration, or by bubbling a less soluble inert gas (helium) through the mobile phase, particularly at higher temperatures (35°C).

A second method of avoiding outgassing (due to mixing) in the solvent delivery system is to pre-dilute the organic solvent (modified Dial-a-Mix). Instead of

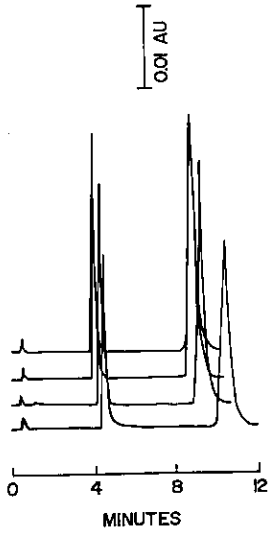


***Figure 1.*** Dial-a-Mix technique using on-line mixing of 55% bottle A (0.1 M acetate) and 45% bottle B (methanol). The first peak is testosterone, the second is corticosterone.

mixing 90% buffer from bottle A with 10% methanol (neat) from bottle B, you could mix 80% of a more-concentrated solution of the same buffer with 20% of 50:50 methanol:water. The methanol:water mixture should be made and filtered at the bench, so some of the effects of mixing are dealt with before on-line mixing occurs. Such techniques are recommended for gradients, and they also will work for Dial-a-Mix mobile phases. Bench mixing of the complete mobile phase is still the recommended procedure, but Dial-a-Mix techniques are convenient for methods development.

The results below demonstrate the erratic performance obtained by on-line mixing of totally aqueous and neat organic solvents (Dial-a-Mix). The performance was improved by bench mixing (isocratic mobile phase) and by pre-diluting the organic solvent (modified Dial-a-Mix).

**Conditions**
System: BAS 200 Liquid Chromatograph

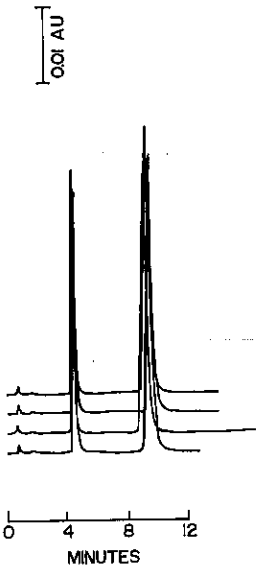


***Figure 2.*** Modified Dial-a-Mix technique using on-line mixing of 50% bottle A (0.11 M acetate) and 50% bottle B (90:10 methanol:water). Note that the acetate concentration was increased to compensate for the extra dilution from bottle B.

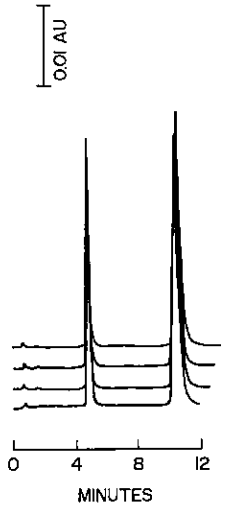


***Figure 3.*** Isocratic technique using bench-mixed mobile phase.

Detector: Variable wavelength UV (240 nm)
Column: 3 µm, C 18 reverse-phase, 100 x 3.2 mm (PN MF-6213)
Temperature: 40°C
Mobile Phase: 55% 0.1 M sodium acetate, pH 7.0, 45% methanol. Flow rate was 1 mL/min.

**Notes**

Peak retention times varied substantially with the unmodified Dial-a-Mix technique (F1). Pressure readings indicated severe fluctuations during these runs, suggesting the presence of air bubbles in the lines.

Both the modified Dial-a-Mix technique, and the isocratic technique, produced good chromatographic results (F2, F3). Pressure readings during these runs were normal.

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