Supplemental Figure 2. A second method of extraction (15,16) confirmed results obtained with the single step extraction method. at-RA and at-ROL were detected in the acidic (345 nm DAD signal) and neutral (325 Dad signal) lipid fractions of postnatal rat olfactory extracts prepared using this alternate method of extraction. 0.3-0.6 g tissue was homogenized in 2 ml PBS, pH 7.4. 5 ml (3:2 hexane/isopropyl alcohol) was added and mixed by vortexing for 2 min. 2 ml sodium sulfate (66.7 mg/ml in water) was added and mixed for 1 min. Samples were centrifuged at 1000 g x 4C x 10 min, the hexane layer was removed, and the aqueous layer was extracted a second time with 5 ml 7:2 hexane/isopropyl alcohol. The organic extracts were pooled, dried down under argon, and resuspended in 1 ml chloroform. The resuspended sample was applied to an aminopropyl bonded silica gel column (500 mg, LiChrosorb or Supelco SPE column) that was equilibrated with hexane. Neutral lipids were eluted with 2 ml 85:15 hexane/ethyl acetate. The column was washed with 2:1 chloroform/isopropanol and then acidic lipids were eluted with 2% acetic acid in diethyl ether. Lipid extracts were dried down under argon and resuspended in HPLC solvent B. Acitretin was included as an internal standard for acidic lipids and retinyl acetate was included as an internal standard for neutral lipids. HPLC runs were extended to 30 min to allow detection of retinyl acetate. Endogenous retinyl esters were not detected using these methods because they are essentially insoluble in Buffer B and because, if present, would elute after the 30 min run time of the HPLC method. The spectrum of the peak labeled at-RA (solid trace, upper left inset) matches that of authentic at-RA (red orange dashed trace, upper left inset) and the spectrum of the peak labeled at-ROL (solid trace, upper right inset) matches that of authentic at-ROL (orange dashed trace, upper right inset).