# **Highly sensitive LC-MS/MS** analysis of **epinephrine** and **norepinephrine** in **plasma** with a measuring range covering the entire normal range

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### *Background*

We present here a highly sensitive LC-MS/MS method for selective analysis of epinephrine (E) and norepinephrine (NE) in plasma samples using an in-sample ion-pairing chromatography technique with 1-heptane sulfonate (HSA) as ion-pairing reagent.

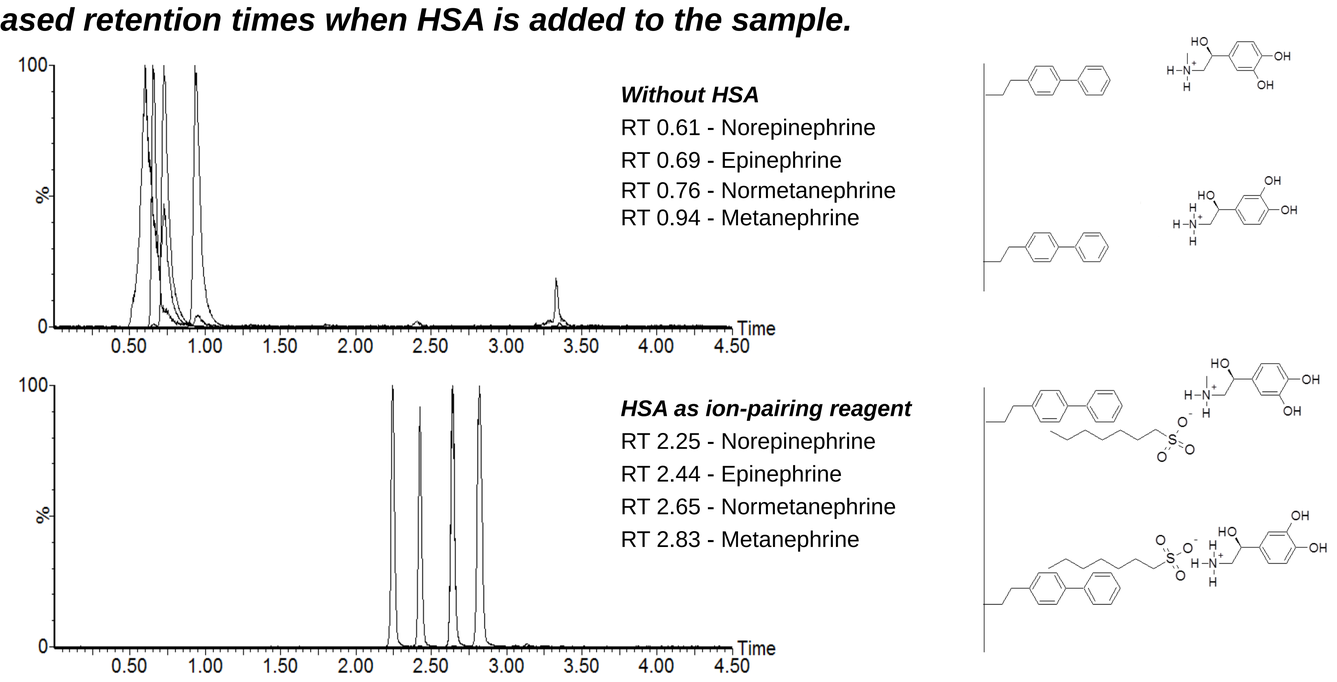
### *Methods*

Plasma catecholamines were extracted by using a Sep-Pak Alumina B, 100 mg 96-well extraction plate (Waters) with d6-norepinephrine (d6-NE) and d3-epinephrine (d3-E) as internal standard, evaporated and reconstituted in 250 µL 35 mM HSA solution.
40 µL were injected on a Kinetex core-shell biphenyl column (100 x 2.1 mm, 2.6 µm) with 0.1% formic acid in water/methanol as mobile phases.
HSA was chromatographically separated from E and NE, allowing HSA to be diverted to waste instead of entering the mass spectrometer ion chamber.

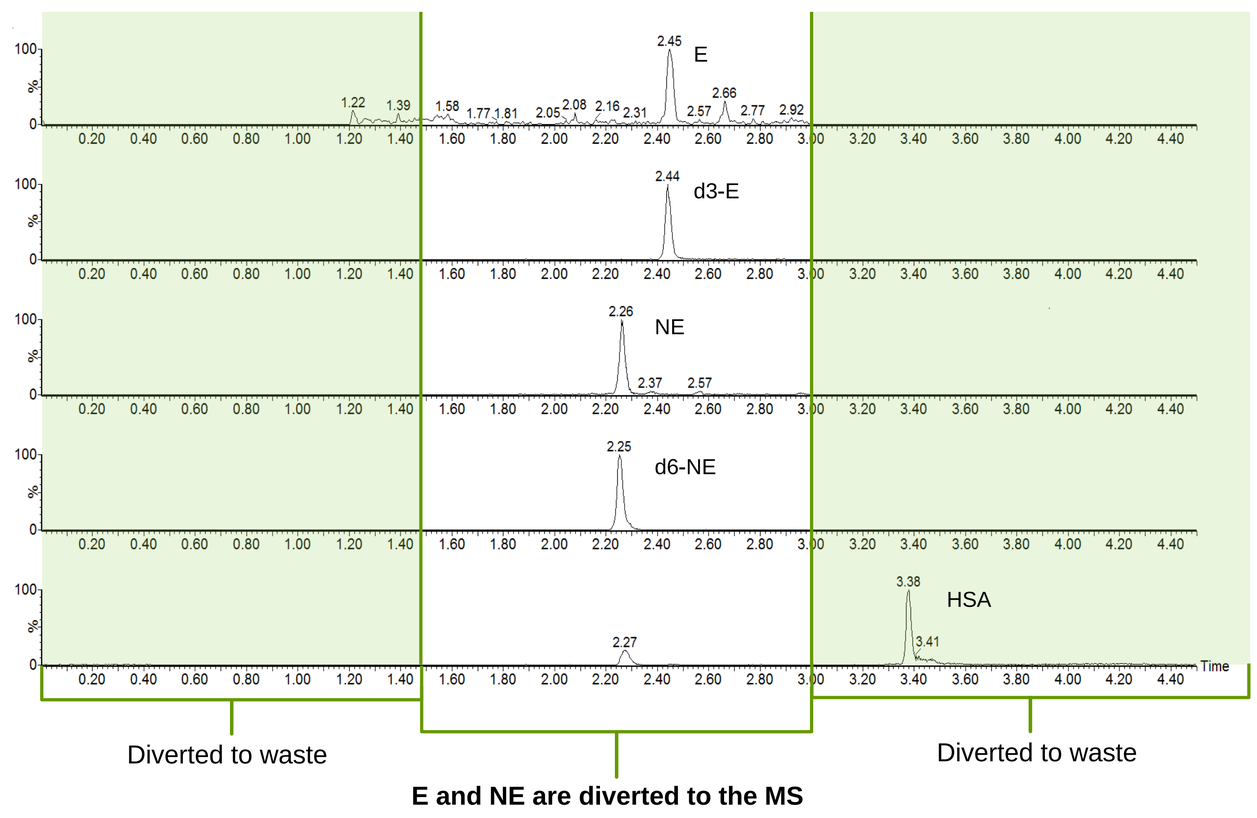
### *Results*

The method vas validated and the limit of quantification (LOQ) was found to be 0.20 and 0.02 nmol/L for NE and E, respectively.
Intermediate precision for control samples in the normal (0.4 and 1.6 nmol/L for E and NE) and abnormal (5.5 and 7.3 nmol/L for E and NE) range, were below 7.5 % for both E and NE.
Mean apparent recoveries over three concentrations were found to be 101% for E and 97% for NE, with CV% of 5% and 7%, respectively.
Quantitative matrix effects investigations showed mean ion suppression of 23% and 7% for E and NE, respectively.
A patient sample comparison of 25 patient samples with Labmedicin Skåne, Klinisk Kemi, Malmø, using a fluorescence HPLC method, displayed a bias of 9.6% for E and -8.4% for NE.

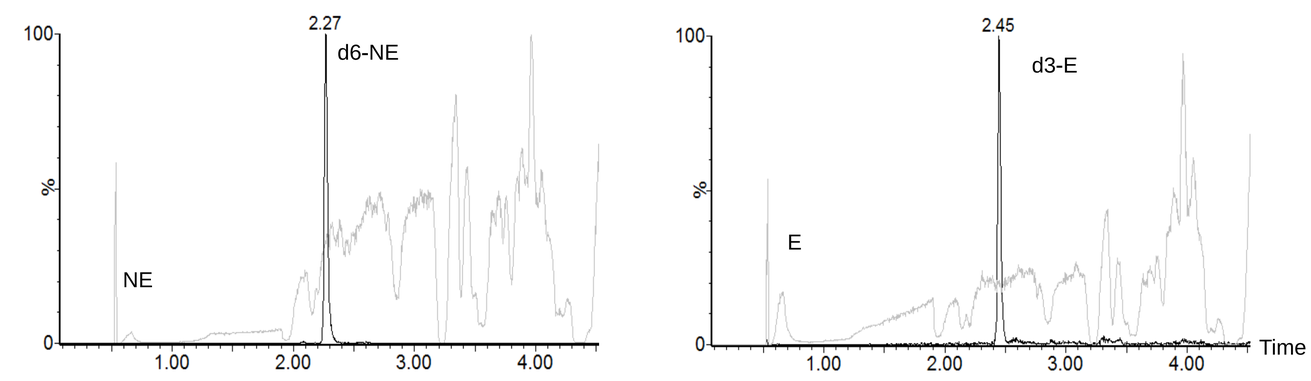
***Increased retention times when HSA is added to the sample.***




***Chromatogram for patient sample containing 0.08 nmol/L E and 0.99 nmol/L NE.***




***Overlaid chromatograms of patient sample and post column infusion of E and NE***

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