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## Analysis of catecholamines in urine by unique LC/MS suitable ion-pairing chromatography

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The catecholamines, epinephrine (E) and norepinephrine (NE) are small polar, hydrophilic molecules, posing significant challenges to liquid chromatography– tandem mass spectrometry (LC-MS/MS) method development. Specifically, these compounds show little retention on conventional reversed-phase liquid chromatography columns. This work presents development and validation of an LC-MS/MS method for determining catecholamines in urine, based on a new approach of ion-pairing chromatography (IPC), in which the ion-pairing reagent (IPR), 1-Heptane Sulfonic Acid (HSA), is added to the extracted samples instead of the mobile phases. A Hamilton STARlet workstation carried out the solid phase extraction of urine samples. The extracted samples were diluted with 60 mmol/L HSA and injected on a Kinetex core-shell biphenyl column with conventional LC-MS/MS suitable mobile phases. Chromatographic separation of E and NE was achieved successfully with very stable retention times (RT). In 484 injections, the RTs were steady with a CV of less than ±4%. Furthermore, HSA was separated from E and NE, allowing HSA to be diverted to waste instead of entering the mass spectrometer ion chamber. The method was validated with good analytical performance, and even though the analysis for urinary catecholamines is increasingly being replaced by plasma free metanephrines in diagnosing pheochromocytomas, this work represents the application of a new analytical technique that can be transferred to other small polar molecules, that are difficult to chromatograph on traditional reversed phase columns..

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