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Determination of highly polar catecholamine with liquid chromatography–tandem mass spectrometry using weak cation-exchange stationary phase to increase retention time

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Abstract

This paper reports method development and validation work to determine highly polar bases, catecholamine compounds, using weak cation-exchange liquid chromatography of low ionic strength mobile phase with electrospray tandem mass spectrometry. Catecholamine compounds, such as epinephrine and norepinephrine, well-known biomarkers to diagnose hypertension disease, spiked in saline solutions are purified with solid phase extraction (SPE) using alumina powders. The extracts are loaded into a weak cation-exchange liquid chromatographic column via an injection loop and analyzed with electrospray-mass spectrometer. The de-salted extracts contain only small amounts of electrolytes to avoid saturating weak cation-exchange sites in the stationary phase with sodium ions. Using carefully selected mobile-phase solvents with optimized compositions (acetonitrile and water 10:90 v/v) and with dilute acid additives (acetic acid 0.1% v/v), we are able to elute catecholamine at sufficient retention times to avoid co-elution of saline matrix residues while maintaining adequate electrospray ionization efficiency of these compounds. Using epinephrine and norepinephrine standards, these methods are validated at the range of 5 to 500 ng mL^{-1}. The measurement accuracy and precision of using epinephrine standards are within 12% and 5.3% respectively, whereas the accuracy and precision are within 6.0% and 4.2% respectively using epinephrine standards.

The detection limits of epinephrine and norepinephrine are 0.10 ng mL^{-1} and 0.45 ng mL^{-1} respectively. The recovery percentages of our solid phase extraction methods using alumina powders are higher than 74%. When the validated calibration curves are used to determine epinephrine and norepinephrine in rat blood dialysates, the determination errors of accuracy and precision are both within 4%, while the determination errors are within 3% in rat blood plasma samples.
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1. Introduction

Liquid chromatography–tandem mass spectrometry (LC–MS–MS) technologies have revolutionized chemical and biological sample analysis [1–3], especially with electrospray ionization methods. Multiple-reaction-monitoring (MRM) technique of tandem mass spectrometry provides tremendous selectivity improvements by reducing noise levels in the ion signal [4]. Liquid chromatography–tandem mass spectrometry instrumentation is also compatible with high throughput automated ancillary techniques such as liquid–liquid extraction and solid phase extraction with well-plate formats as well as methods using on-line extraction following direct sample injection [5]. The high throughput compatibility of LC–MS–MS makes this instrumentation suitable for high-speed biological sample analysis and for quantitation of trace impurities in drug products [6]. Most developed methods using commercially available LC–MS–MS are reliable at the 1–10-ng mL^{-1} detection limit.

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High sensitivity and selectivity features of LC–MS–MS work best when the analytes do not co-elute with matrix components from injected samples. These inherent species in the matrix, especially salts, generate a competition in the ion source known as ion suppression [7]. In several cases, it was found that such matrix effects significantly affected the ionization of analytes, thereby diminishing the sensitivity advantages gained in MRM. Most conventional reverse-phase liquid chromatography separations cannot avoid co-eluting highly polar compounds from the sample matrices. Although normal phase liquid chromatography has been widely used to separate polar compounds, these separation approaches have proven to be less convenient than reverse-phase approaches in electrospray LC–MS–MS because of the non-conducting and flammable nature of mobile phases. Due to the limitations of both reverse-phase and normal phase chromatography, there remains a need to develop special separation methods that are compatible with LC–MS–MS and are capable of separating polar compounds that are poorly retained in reverse-phase chromatographic systems.

Several previous approaches propose increasing polar compound retention time. Employing a polar group embed stationary phase with reduced organic content of mobile phase only marginally increases chromatographic capacity. Although adding ion-pairing reagent sometimes sufficiently increases capacity, this method often causes ionization suppression. Other alternatives, such as analyte derivatization methods, usually have difficulty developing universal separation methods. Although ion-exchange chromatography is a well-known strategy for separating polar compounds, especially when their polar functionalities are acidic or basic, this type of separation is usually performed under mobile-phase conditions of high salt concentrations and is, therefore, incompatible with electrospray ionization mass spectrometry.

This paper investigates a specialty analytical column packed with weak cation-exchange liquid chromatography stationary phase that retains highly polar catecholamine compounds, such as epinephrine and norepinephrine, using isocratic elution under electrospray-mass spectrometry compatible mobile-phase conditions.

LC–MS–MS assays using a weak cation-exchange column to determine highly polar compounds, including catecholamine, have not been published elsewhere to our knowledge. Epinephrine and norepinephrine are well-known biomarkers for diagnosing hypertension diseases. These compounds are produced in the medulla of the adrenal gland and work in response to body stress. In addition, they affect many physiological and metabolic activities including heartbeat, nerve response, and muscle activity. Irregular levels of catecholamine released from nerve endings have been suggested as the hypertension development mechanism in humans [8,9] and in spontaneous hypertensive rats [10–12]. Methods using derivative conversion to volatile conjugates for gas chromatography–mass spectrometry determination are reported [19–21] in clinical and animal studies, to assay samples in a more complicated matrix for instance plasma. Radioactive detection methods are also used to determine catecholamine concentrations in biological fluid samples. Although assay sensitivities are not questioned, they are much less convenient than LC–MS–MS methods for conversion to high throughput methods because of their tedious sample preparation procedures, usually performed by well-trained personnel. Conventional LC–MS–MS assays provide moderate detection limits [13–18] even with a less complicated biological fluid matrix such as urines, due to ionization suppression problems of co-eluted matrix species or ion-pairing additives in mobile phase.

Unlike strong cation-exchange liquid chromatography, weak cation-exchange liquid chromatography elutes analytes using low ionic strength mobile phase, similar to that used in reverse-phase liquid chromatography. These mobile-phase solvents, such as water acetonitrile, or their mixtures, are compatible with electrospray ionization when lightly doped with electrolyte additives.

Once we obtain optimized chromatographic conditions compatible with electrospray ionization processes, we develop and validate analytical methods to determine highly polar base catecholamine bases, such as epinephrine and norepinephrine in saline solutions with triple-quadrupole mass spectrometer. Prior to loading catecholamine extracts into chromatographic columns, we purify sample solutions via solid phase extraction using alumina powders. Sufficient injection sample de-salting is required to avoid saturating weak cation-exchange sites with sodium ions in order to main adequate chromatographic separation performance. We also investigate the determination differences of these compounds between saline, blood microdialysate, and plasma samples. The detection limits of our methods are below 1 ng mL^{-1}.

2. Experimental

2.1. Materials

Catecholamine compounds, including epinephrine and norepinephrine are acquired from Sigma (St. Louis, MO, USA). Internal standard compound isoproterenol is also from Sigma. Glacial acetic and formic acids (Mallinckrodt Baker Inc.; Paris, KY, USA) are diluted to prepare mobile-phase and chromatography injection solvents with organic solvents (all from Mallinckrodt), acetonitrile, methanol, or isopropyl alcohol and water. Reagent grade water (Mallinckrodt) is used for dilution. The stock solution of saline is NaCl at 0.85% (0.14 M). Blank blood dialysate and plasma are collected from Sprague–Dawley rats.

2.2. Apparatus

Weak cation-exchange columns (WCX; 5 μm; 150 mm × 2.1 mm) are obtained from Diazem Corporation (Midland, MI, USA). The WCX media is prepared with 120-angstrom HPLC grade 5-μm silica. The carboxylate cation-exchange functionality on the surface of the silica was derived from a saponified carbomethoxy silane. The silica is packed into an Isolation Technology modular column using standard slurry packing techniques. Triple-quadrupole mass spectrometer used to perform multiple-reaction-monitoring (MRM) technique in this work is Quattro

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