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# Minimization of ion suppression in LC–MS/MS analysis through the application of strong cation exchange solid-phase extraction (SCX-SPE)

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**Abstract**

Ion suppression of drug response is a major source of imprecision for bioanalytical analysis using LC–MS/MS. Endogenous phospholipids cause ion suppression in both positive ESI and negative ESI modes and must be removed or resolved chromatographically. Three types of ion-exchange solid-phase extraction mediums were evaluated to determine their abilities to remove phospholipids. It was determined that although mixed mode phases fulfills the requirements of retaining both analytes and diverse metabolites, reverse phase retention mechanisms are detrimental in eliminating ion suppression caused by late eluting phospholipids. If an analyte and its metabolites can be retained using an ion-exchange mechanism alone, mixed mode extraction phases should be avoided.


*Keywords:* LC–MS/MS; Ion suppression; SPE; SCX-SPE; Mixed mode SPE; Phosphatidylcholine; LysoPC

## 1. Introduction

Today, liquid chromatography and tandem mass spectrometry (LC–MS/MS) is the method of choice for the quantitation of analytes in biological matrices. The combination of LC and MS/MS offers unparalleled sensitivity and specificity few other techniques can match [1,2]. It comes as no surprise that for a period of time following the introduction of LC–MS/MS instrumentation, sample cleanup and high-resolution chromatography were considered superfluous [3,8]. With their importance deemphasized, sample cleanup typically involved acetonitrile precipitation of plasma proteins and chromatographic separation was achieved by the use of short analytical columns (typically 3 cm and less) with steep gradients or short isocratic runs, often less than 2 min. However, it became apparent that inadequate sample cleanup and chromatography often led to ion suppression that caused irreproducible results for some bioanalytical methods [4,5]. The effect of ion suppression is observed primarily in electrospray ionization (ESI) whereby analyte signal is attenuated by competition from the ionization of bulk ions inside the solution droplets [15,16]. Although these disturbances are not visible in the analyte MRM channels of interest, the precision and accuracy of bioanalytical methods can be compromised by sample-to-sample differences in matrix ion concentrations and components competing with the ionization of analyte. A number of techniques to visualize ion suppression have been proposed in the literature. The most common method involves a post-column infusion of the compound of interest while the MRM transition is recorded during an injection of blank matrix sample [7], producing chromatograms similar in appearance to IR spectra, ion suppression is revealed as dips in the constant background signal of analyte.

Although solvent additives and mobile phase components can cause ion suppression, their impact is relatively insignificant when compared to ion suppression generated by the presence of endogenous materials in biological samples;

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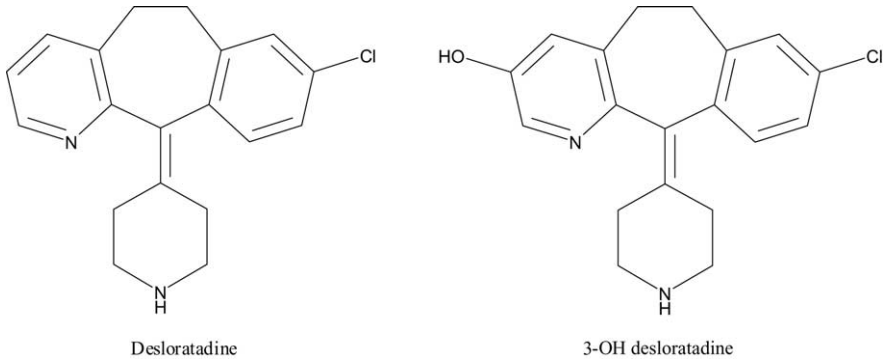


Fig. 1. Structure of desloratadine and 3-hydroxy desloratadine.

i.e., matrix effects [6]. Indeed, matrix effects predominate as the cause of ion suppression. Of all the components in plasma, lipids are the most variable. Both the type and quantity of lipids found in plasma fluctuate within individuals and differ from person to person are primarily dependent on diet and metabolic rate. However, despite significant lipid variation, phosphatidylcholine (also know as 'lecithin') is the principle phospholipid circulating in the plasma [9]. As a zwitterion, phosphatidylcholine can cause ion suppression in both positive ESI and negative ESI modes because of its ability to ionize in both environments. In a recent study, serum albumin and phosphatidylcholine were determined to be the major causes of ionization suppression in analysis of verapamil following protein precipitation and reverse phase solid-phase extraction sample cleanup [10]. Therefore, the removal of phospholipids as a principle agent of ion suppression is an extremely important component of any extraction process. Several techniques have been applied toward the removal of phospholipids in order to obtain cleaner sample extracts [16]. In one such study, a modified lanthanide column was used successfully to remove the bulk of the phospholipids through a two-step cleanup process involving liquid/liquid extraction with methyl *tert*-butyl ether (MTBE) followed by solid-phase extraction on a proprietary column [11]. Although effective, this two-step cleanup process is time consuming and difficult to automate. The goal of our study was to evaluate popular and commercially available ion exchange and mixed mode solid-phase sorbents and propose a simpler procedure for removing phospholipids without impacting the recovery of compounds of interest. To this end, we evaluated three leading solid-phase extraction sorbents available in a 96-well format for their ability to extract the model compounds such as desloratadine and 3-hydroxy desloratadine while removing phospholipids.

## 2. Experimental

### 2.1. Materials

Desloratadine with a purity of 98.3%, and 3-hydroxy desloratadine with a purity of 99.3% were synthesized at Schering-Plough Research Institute (Kenilworth, NJ) (Fig. 1). Human plasma with EDTA as the anticoagulant was purchased from Bioreclamation Inc. (Hicksville, NY). All other chemical reagents were purchased from either Fisher Scientific (Fair Lawn, NJ) or Sigma–Aldrich Co. (St. Louis, MO). All chemical reagents were either OPTIMA® Grade or Certified ACS Grade unless otherwise noted.

### 2.2. Instrumentation

A Sciex API 3000 (Applied Biosystems, Ont., Canada) mass spectrometric system equipped with a TurboIonspray™ interface was used as the detector. Unless otherwise noted, this mass spectrometer was operated in the multiple reaction monitoring (MRM) mode using positive ion electrospray. Desloratadine was monitored with a MRM transition of *m/z* 311 → *m/z* 259 while 3-hydroxy desloratadine was monitored with a MRM transition of *m/z* 327 → *m/z* 275. The following are the API 3000 instrument parameters used for all experiments: nebulizer = 10, curtain gas = 10, collision energy = 30 eV, ion spray voltage = 4000 V, turbo heater temperature = 400 °C. The chromatography system consisted of a pair of Shimadzu (Shimadzu Corporation, Columbia, MD, USA) 10ADvp LC pumps controlled via a Shimadzu SCL-10A system controller. A Shimadzu DGU-14A degasser is standard on this system. Sample injection was automated via a CTC PAL autosampler. Data collection and peak integration were performed using Analyst™ 1.3.1 software.

### 2.3. Solid-phase extraction disks

Three 96-well solid-phase extraction disks were evaluated. The first is commercially available from Waters Corporation under the trade name Oasis® HLB MCX. Based on a water wetable polymeric backbone, the 96-well Oasis® HLB MCX plate has dual mode functionality using a sulfonic acid moiety as its strong anionic functional group and a copolymeric backbone for retention by hydrophobic and hydrophilic mechanisms. The 96-well solid-phase extraction plate used for this experiment had a total of 10 mg of sorbent.