ABSTRACT:

The purpose of this project was to develop a rapid and simple high throughput quantitation method with minimal sample preparation. The ability to maintain a 1 mL/min flow during the analytical portion of the run allows for a very rapid 0.5 minute gradient elution and high sample throughput. Conventional LCMS equipment provides optimal sensitivity at less than 0.5 mL/min, splitting of the analytical flow is required at higher flow rates resulting in loss of sensitivity. Utilizing the API 4000 Turbo V source with direct injection of the entire 1 mL/min analytical flow, provides excellent sensitivity and rapid LC gradient elution of analytes as well as increased sample throughput three fold.

RESULTS:

Figure 1. Compound Structures

Figure 2. API 4000 Turbo V sources

Figure 3. HPLC Configuration

Figure 4. MS Method

Figure 5. HPLC Method

Figure 6. 62P Femtograms on Column, Plasma Precipitation on API 3000, 4 Minute Analysis Time

Figure 7. 62S Femtograms on Column, Plasma Precipitation on API 3000, 4 Minute Analysis Time

Figure 8. Standard Sample Curve for Three Analytes 272 Plasma Samples Injected

MATERIALS AND METHODS:

Samples were prepared by a 1:1 dilution with 10% formic acid in water: standards were spiked into the 10% formic acid:water solution before combination with the plasma. A 2 micron filter was used as a pre-column to prevent clogging of the Oasis column. A total of 272 plasma samples were injected in this batch. The HPLC system consisted of an HPLC1100 binary analytical pump and a Yoke valve by Yoke (Figure 3). The sample valve was a 6-port, 100V, with a 10uL loop (Figure 4). Prior to loading the plasma samples, the analytical pump was used for injections, a C HPLC internal valve was added to the CTO to reduce cross over. An Applied Biosystems/SCIEX API 4000 triple quadrupole mass spectrometer provided MS/MS detection. The HPLC gradient is outlined in Figure 5, solvent A consisted of water 0.1% formic acid and 10 mM ammonium acetate, solvent B consisted of acetonitrile with 0.1% formic acid. Total analysis time was about 1.2 minutes per sample. A/Sample loading time was sufficient for column equilibration. An Oasis HLB 25 mm x 2.1 mm extraction cartridge was used for sample extraction and gradient elution. During the first 0.4 minutes of the run, the sample was loaded and washed at 2 mL/min with the sample valve diverting to waste. After 0.4 minutes, the flow rate was dropped to 1 nL/min and a standalone gradient was employed for sample elution.

Figure 8 contains all the TICs produced, as well as the MS/MS transitions data. All analytes were obtained by automatic tuning of the Turbo V source. The Turbo V source was tuned manually by FIA and the following conditions were determined to be optimal for the solvent composition and flow rate: Curran gas (g) = 10, ISF gas = 100, Cad gas = 7. API 3000 samples were created by Online/Offline precipitation of rat plasma with standards spiked 1:1 with extract. A standard water, Acetonitrile:0.1% formic acid 200 uL/g Niger was used with a Restek Asaplex 2.2, 5u mm column. Overall LCMS total run time for this method was around 4 minutes.

CONCLUSIONS:

Linearity of nearly three orders of magnitude with acceptable correlation coefficients (Figure 1). The major limiting factor for the upper end of the concentration curve is contamination from the Oasis column. The accuracy of all of the standards are excellent for all three analytes (Figure 2). The plasma precipitation method has less carryover when samples run over or over three orders of magnitude are injected. The CVs in Figure 2 are adequate (less than 10%) across the concentration range for all three analytes. Better CVs (less than 7%) are obtained by the traditional plasma purification method. Use of a 3 mL/min flow rate reduces turn time by about 1/3. Sample throughput was increased 2 fold with the use of two columns, at least twice the number of samples could be run before pressure limitations become critical. Use of the API 4000 Turbo V source in Electrospray mode allowed for excellent sensitivity at 1 mL/min. Due to extraction limitation, detection limits for most compounds are greatly reduced using online extraction. The improved sensitivity, better deionization and gas focusing in the API 4000 source results in a smaller sample volume. The API 4000 Turbo V source allows for direct injection of the entire 1 mL/min analytical flow, providing excellent sensitivity and rapid LC gradient elution of analytes as well as increased sample throughput three fold.

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