DETERMINATION OF PHARMACEUTICALS BY CAPILLARY HPLC-MS/MS

S.V. CALUGARU1[1] | E.P. GATES[1] | W.R. WEST[1] | M.L. LEE[1] | R. FRANCESCHINI[2]

[1]**Axcend LLC**, Provo, UT 84604, UNITED STATES

[2]**SRA INSTRUMENTS SPA**, Via alla Castellana, 3 - 20063 Cernusco sul Naviglio, ITALY

INTRODUCTION

The widespread use of pharmaceuticals including hormones, antibiotics, non-steroidal anti-inflammatory drugs, antidepressants, and antifungal agents results in significant discharge of these drugs and their metabolites into wastewater. Concerns have been raised over their potential to contaminate ground and surface waters, and to enter drinking water supplies. Therefore, monitoring pharmaceuticals in aqueous samples represents an important analytical task. Using capillary LC for this purpose provides many advantages including dramatically reduced consumption of toxic and expensive organic solvents and potentially higher sensitivity when using MS detection resulting from intrinsically low mobile phase flow rates.

EXPERIMENTAL DETAILS

INSTRUMENTATION

A compact capillary LC, the Axcend Focus LC with 2.2.0 Axcend Drive software (Axcend, Provo, Utah, USA), was used in this study. The LC was interfaced to a triple quadrupole mass spectrometer, the Ultivo LC/TQ G6465B equipped with Jet Stream Electrospray Ionization source (Agilent Technologies, Santa Clara, California, USA). MassHunter software (Acquisition: v1.1, Qualitative Analysis: v10.0, Quantitative Analysis: v10.0) was used for MS control and data processing. To accommodate the capillary LC mobile phase microflow rates, the regular nebulizer of the ion source was replaced with a microflow nebulizer (Part No G1946-67260) obtained from Agilent. A 25 cm long, 360 µm OD, 25 µm ID PEEKsil tubing (Part No. 0624374, Trajan, Melbourne, Victoria, Australia) served as a transfer line. One end of this tubing was attached to the solvent outlet from the column cartridge using a PEEK zero-dead-volume 360 µm union with a 50 µm bore hole (Part No. C360UPK2, VICI Valco Instruments, Houston, Texas, USA). The other end of the tubing was connected to the microflow nebulizer using a stainless steel 1/16" to 360 µm zero-dead-volume reducing union with a 100 µm bore hole (Part No. C360RUS64, VICI Valco Instruments). Direct sample infusion into the mass spectrometer for optimization of acquisition parameters was performed with a Model 22 syringe pump from Harvard Apparatus (Holliston, Massachusetts, USA).

CHEMICALS AND SOLVENTS

Mixtures of pharmaceuticals (Mix #1 containing acetaminophen, caffeine, carbamazepine, ciprofloxacin, erythromycin, fluoxetine, sulfamethoxazole, and trimethoprim at 200 µg/mL each in methanol, and Mix #2 containing gemfibrozil, ibuprofen, naproxen, and triclosan at 200 µg/mL each in methanol) were purchased from Restek (Bellefonte, Pennsylvania, USA). Calibration standards were prepared by serial dilutions of these test mixtures in water. D4-Acetaminophen (100 µg/mL in methanol) and D10-carbamazepine (100 µg/mL in methanol) were purchased from HPC Standards (Atlanta, Georgia, USA) and used as internal standards in Mix #1 samples. D3-Ibuprofen (100 µg/mL in methanol) was purchased from Cerilliant (Round Rock, Texas, USA) and used as an internal standard in Mix #2 samples. LC-MS grade solvents (water and acetonitrile) were obtained from Sigma-Aldrich (MilliporeSigma, St. Louis, Missouri, USA) and LC-MS grade formic acid was purchased from ThermoFisher Scientific (Waltham, Massachusetts, USA).

HPLC METHODS

Mix #1 was analyzed using an HSS T3 Acquity UPLC column (10 cm x 150 µm i.d., 1.8 mm particle size) from Waters (Milford, Massachusetts, USA). A small diameter flow-cell detector was used for monitoring UV absorption at 275 nm. Mix #2 was analyzed using a C18 column (10 cm x 150 µm i.d., 1.7 mm particle size) from CoAnn Technologies (Richland, Washington, USA). UV absorption was monitored at 235 nm using an on-column detector. In both LC methods, solvent A was composed of 97% water, 3% acetonitrile, and 0.1% formic acid, and solvent B was composed of 97% acetonitrile, 3% water, and 0.1% formic acid. The flow rate was 1 µL/min and the injection volume was 250 nL (full loop). For the separation of Mix #1 components, the content of solvent B in the mobile phase was 3% during the initial 0.5 min after sample injection, then it was linearly ramped to 26% over 1.5 min, held at 26% for 5 min, linearly ramped to 97% over the next 3 min, and finally it was held at 97% for 5 min. For Mix #2 the content of solvent B in the linear mobile phase gradient was 3% for the initial 0.5 min, then it was increased to 60% over 1 min, to 77% over the next 2.5 min, to 97% over the following 1.5 min, and finally it was held at 97% for 3 min.

MS METHODS

Multiple reaction monitoring (MRM) was used in positive and negative polarity modes for the analysis of both test mixtures. Deuterated acetaminophen, carbamazepine and Ibuprofen were used as internal standards. The MRM methods were optimized using MassHunter optimizer software by injecting the respective diluted test mixture (each component at 100 ng/mL) into the ion source at a flow rate of 1 µL/min with a syringe pump. The identified optimum fragmentor voltage and collision energy values for the quantifier and qualifier ions of the individual analytes and internal standards are presented in Table 1 for Mix #1 and in Table 2 for Mix #2. The other MS parameters were as follows: capillary voltage, 3000 V; gas temperature, 200 oC; gas flow rate, 5 L/min; nebulizer pressure, 10 psi; and dwell time, 50 ms except for fluoxetine and sulfamethoxazole, which was 100 ms.

RESULTS AND DISCUSSION

IDENTIFICATION OF ANALYTES

Two transitions between the parent ion and the product ions were selected for monitoring analytes and internal standards. The first transition was used for analyte quantification and the second one for qualification (Tables 1 and 2). No fragmentation of triclosan was observed under the tested conditions. For this pharmaceutical, only the molecular ion was monitored (Table 2). The chemical structures of the analyzed pharmaceuticals are shown in Figure 1 for Mix #1 and Figure 2 for Mix #2. The chromatographic separations of the components of Mix #1 and Mix #2 with UV detection at 275 nm and 235 nm are presented in Figures 3 and 4, respectively. Each analyte in the UV chromatograms was identified based on MS data. The extracted ion chromatograms for Mix #1 and Mix #2 are shown in Figures 5 and 6, respectively.

QUANTIFICATION OF ANALYTES

To normalize the intensities of the MS signals from Mix #1 components, deuterated analogs of acetaminophen (D4-acetaminophen) and carbamazepine (D10-carbamazepine) were used as internal standards at a final concentration of 500 ng/mL each. D4-acetaminophen served as an internal standard for acetaminophen and ciprofloxacin, while D10-carbamazepine was used for the other compounds in Mix #1. Linear calibration curves with a regression coefficient $R^2 > 0.99$ were obtained in the following concentration ranges: 30-1,000 ng/mL for carbamazepine, 50-10,000 ng/mL for trimethoprim, 100-10,000 ng/mL for acetaminophen, 100-5,000 ng/mL for caffeine, 300-10,000 ng/mL for fluoxetine, 300-3,000 ng/mL for sulfamethoxazole, and 500-10,000 ng/mL for erythromycin. Quadratic regression was used to obtain calibration curves with a regression coefficient $R^2 > 0.99$ in the concentration range of 500-10,000 ng/mL for ciprofloxacin. Typical calibration curves are shown in Figures 7 and 8 for acetaminophen and carbamazepine, respectively. In the case of Mix #2, D3-ibuprofen was used at a final concentration of 250 ng/mL as an internal standard for all analytes. Calibration curves with a regression coefficient $R^2 > 0.99$ were obtained in the following concentration ranges: 30-10,000 ng/mL for gemfibrozil, ibuprofen, and naproxen (linear regression), and 30-1,000 ng/mL for triclosan (quadratic regression). Representative calibration curves are shown in Figures 9 and 10 for ibuprofen and naproxen, respectively.

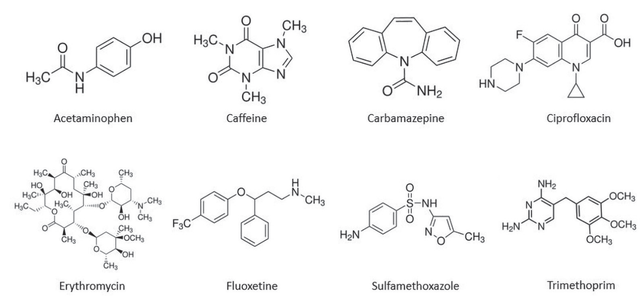

Figure 1: Chemical structures of the compounds in Mix #1.

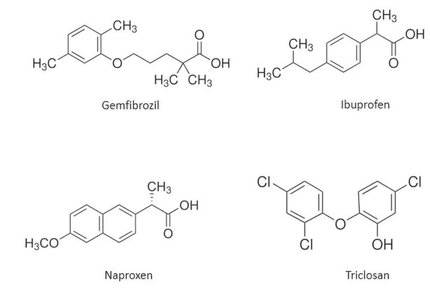

Figure 2: Chemical structures of the compounds in Mix #2.

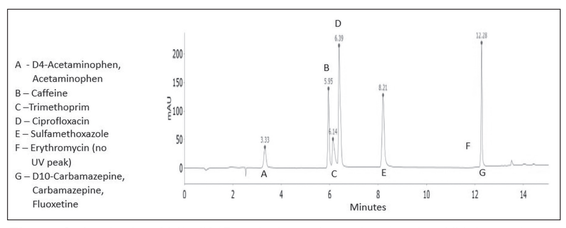

Figure 3: Analysis of Mix #1 (5 µg/mL each analyte) using UV detection at 275 nm. Identifications are based on MS data (see Figure 5).

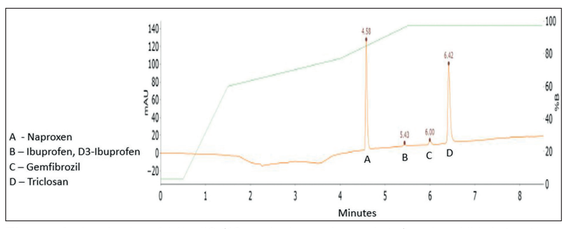

Figure 4: Analysis of Mix #2 (10 µg/mL each analyte) using UV detection at 275 nm. Identifications are based on MS data (see Figure 6).

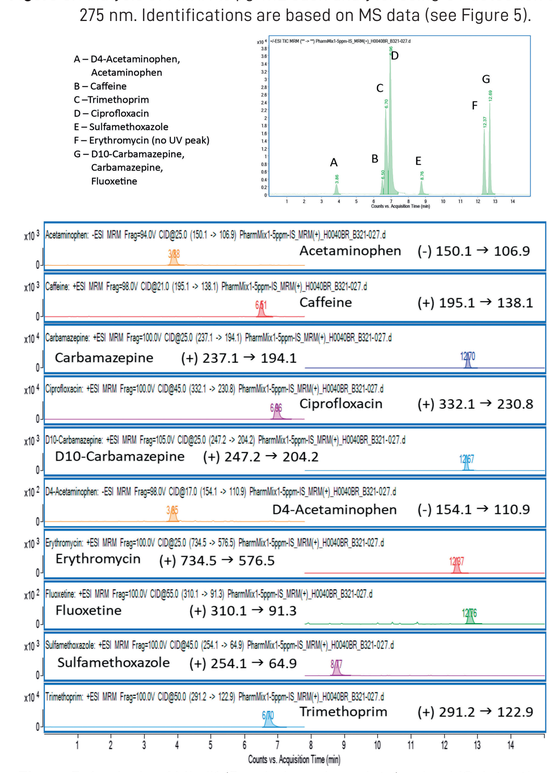

Figure 5: Analysis of Mix #1 (5 µg/mL each analyte) using MS detection in the MRM mode: total ion current (top) and extracted ion chromatograms (bottom).

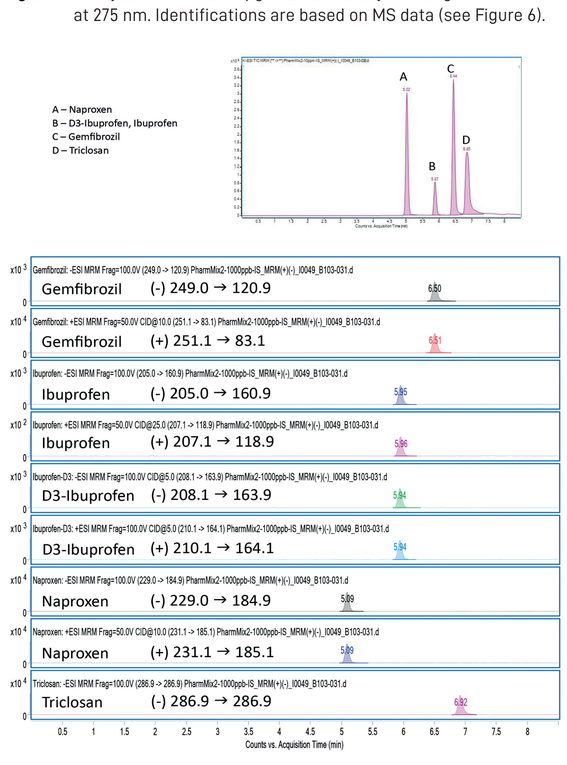

Figure 6: Analysis of Mix #2 (10 µg/mL each analyte) using MS detection in the MRM mode: total ion current (top) and extracted ion chromatograms (bottom).

CONCLUSIONS

Identification and quantification of pharmaceuticals in mixtures of components in model aqueous samples based on capillary HPLC coupled to tandem MS was successfully performed.

Calibration curves with a regression coefficient R^2 above 0.99 were obtained in the concentration ranges typically covering over two orders of magnitude (for example, 30-10,000 ng/mL for acetaminophen).

The developed HPLC-MS/MS methods can serve as a basis for further development of quantitative capillary HPLC-MS/MS determination of pharmaceuticals in biological matrices.

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