Identification and Determination of Carisoprodol in Tablets by Liquid Chromatography/Mass Spectrometry

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ABSTRACT: A method for the identification and determination of carisoprodol in tablet dosage form is described. Tablets were ground and carisoprodol was extracted using acetonitrile with sonication. Extracts were filtered and further dilutions were made with water. The identification of carisoprodol was accomplished using a single quadrapole mass spectrometer coupled to a liquid chromatograph with an electrospray source and positive ion detection. For determining the carisoprodol content, selected ion monitoring of the molecular ion was used. A 5 µm 2.1 x 150 mm Zorbax SB-C18 column and a mobile phase of 35 % acetonitrile (0.1 % formic acid) and 65 % water (0.1 % formic acid) at a flow rate of 0.4 mL/minute provided adequate retention. The calibration curve generated during this analysis was linear between 0.5 and 40 µg/mL for carisoprodol with a correlation coefficient, $r \geq 0.9996$. Spikes of tablets gave an average recovery of 93 % for carisoprodol.

KEYWORDS: Carisoprodol, Meprobamate, Liquid Chromatography/Mass Spectrometry, ESI-LC/MS, Forensic Chemistry

Introduction

Carisoprodol is a centrally acting muscle relaxant, with analgesic properties[1], making it a popular drug of abuse. Carisoprodol is widely available for purchase on the internet with or without a prescription. It is also available in combination with other analgesics, such as aspirin and codeine. Within the body, carisoprodol is metabolized to meprobamate (Figure 1), an anti-anxiety agent prescribed primarily to treat anxiety, tension, and associated muscle spasms. Meprobamate's onset and duration of action are similar to the intermediate-acting barbiturates; however, therapeutic doses produce less sedation and toxicity than barbiturates. This conversion may account for some of the properties associated with carisoprodol. These barbiturate-mimicking properties likely contribute to its abuse[2].

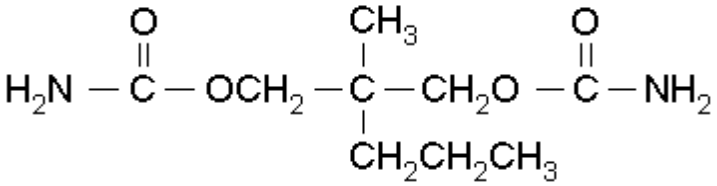

Figure 1: Meprobamate

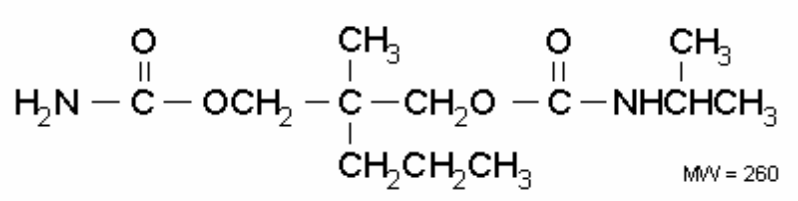

Figure 2: Carisoprodol

The structure of carisoprodol (Figure 2) is such that it does not have a UV chromophore with significant absorbance. Therefore, the USP Assay method for Carisoprodol Tablets employs a liquid chromatograph equipped with a refractive index detector[3]. Our laboratory does not currently have an operational refractive index detector, and numerous literature searches resulted in few references to carisoprodol analysis. We performed analysis of the carisoprodol tablets using a liquid chromatographic (LC) separation similar to that in the USP, but with mass selective (MS) detection. This paper describes the mass spectral identification of carisoprodol and the determination of the carisoprodol content of five individual tablets, each containing an unknown amount of carisoprodol.

Experimental

Apparatus

1. LC-MS System: Agilent (Agilent Technologies, Atlanta, GA) 1100 series LC-MSD with an electrospray source. Chemstation G1701AA version A.09.01 was used for data acquisition and processing.
2. Analytical column: Zorbax SB-C18, 2.1 x 150 mm, 5 mm (Agilent Technologies, Part # 883700-922).
3. Syringe filters: 25 mm diameter 0.45 µm Nylon syringe filters (National Scientific, Catalog #F2500-1), or equivalent.

Materials

1. Mobile phase: 35 % acetonitrile (0.1 % formic acid) and 65 % water (0.1 % formic acid). A liter of each was prepared by adding 1 mL of formic acid (88 % A.C.S. reagent, Aldrich Chemical Company, Milwaukee WI, Catalog #39, 938-8) to each respective solvent.
2. HPLC grade acetonitrile and DI water.
3. USP reference standard, Carisoprodol (Lot F). A stock standard was prepared at approximately 2 mg/mL in acetonitrile. Working standards were prepared by serial dilution with DI water at 40 µg/mL, 20 µg/mL, and 10 µg/mL.

Sample Preparation

Preparation of the carisoprodol tablets consisted of grinding five (5) individual tablets with a mortar and pestle into a fine powder. Each of the individual ground tablets was transferred into an individual 20-mL glass scintillation vial. To each vial, 10 mL of acetonitrile was added and the solutions were sonicated for 15 minutes. A portion of each solution was passed through a 25 mm 0.45 µm nylon syringe filter. Based on internet searches[1], the tablets were suspected of containing 350 mg carisoprodol each; therefore an additional dilution was necessary to decrease the filtrate concentration into a range suitable for MS detection. The filtrate was further diluted by taking a 100 µL aliquot, adding 10 mL DI H_2O, mixing, and then taking 100 µL of this solution to 1 mL with DI H_2O.

Method Validation – LC/MS Assay

Each of two individual tablets was spiked at a different level with a portion of the USP Carisoprodol reference standard. After grinding a single tablet, a known quantity of solid carisoprodol standard equivalent to less than that expected to be present, was added. A second tablet was ground and spiked with a portion of standard in excess of that expected to be present in the tablet. The acetonitrile was added, and the solutions were treated the same as the sample solutions, with the exception of an additional dilution. To bring the spike preparation into the calibration curve range, the final dilution was 30 µL of the spike solution diluted to a total volume of 1 mL with DI water.

Linearity of carisoprodol was established from five separate standards ranging from 0.5 µg/mL to 40 µg/mL. This concentration range was chosen to bracket the diluted sample concentrations. The plot of peak area versus concentration was linear, and the correlation coefficient, r, for carisoprodol was calculated to be 0.9996.

The limit of detection (based on signal:noise of 10:1) was determined for carisoprodol by analysis of a low level standard. The noise level was calculated from the average of ten blank injections, using the area response within the retention time window corresponding to carisoprodol. The detection limit for carisoprodol, on column, was 6.7 picograms.

LC/MS System

The electrospray interface was operated in positive ion scan mode with a mass range of 90-350 amu. The internal capillary voltage was set at 3000 volts. The nitrogen drying gas flow rate used was 10 L/min at 300^{O} C, and the nebulizer pressure was set at 20 psig. For the initial screening, the MS was also operated in the full scan mode with a mass range of 90-350 amu. For the determination of carisoprodol content, the instrument was switched to selected ion monitoring (SIM) mode for more sensitivity and better peak shape. Conditions were set to monitor the protonated molecular ion at m/z 261 $[M + H]^{+}$.

The mobile phase consisted of 35 % acetonitrile and 65 % DI water (both with 0.1 % formic acid), pumped through a C_{18} column at 0.4mL/min. The column thermostat was set at 25^{O} C, the run time was 8 minutes, and 1.0 µL injections were made for all samples and standards.

Data Treatment

Total ion chromatograms were generated for all samples, spikes, standards, and blanks. Each chromatogram was integrated at the retention time corresponding to the retention time of the peak observed in the carisoprodol standard. Peaks that were observed in the blank chromatograms in the retention time range of the carisoprodol peak were small enough that their contribution to the sample peak area was considered negligible. Quantitation of carisoprodol was performed using the data obtained from the SIM ion chromatograms.

Results and Discussion

Initial method development for this work included use of methanol (0.1 % formic acid) in place of acetonitrile in the mobile phase, as well as use of UV detection. However, acetonitrile was chosen for the organic component of the mobile phase because it resulted in increased retention and improved peak shape for carisoprodol. Ideally, carisoprodol would generate an adequate UV signal for determining the carisoprodol content. Based on its structure, it is not expected to absorb at 280 nm or at 214 nm. UV experiments verified no absorbance from this compound, even at 20 times the injection volume used for MS detection. Therefore, quantitation was performed based on SIM data generated by the MS.

Spray chamber parameters were optimized for carisoprodol using the flow injection analysis mode of the instrument. In-source collision induced dissociation (CID) generated fragment ions and was accomplished by adjusting the instrument's fragmentor voltage. Optimum CID conditions were obtained by injecting a 20 µg/mL

carisoprodol standard at several fragmentor voltages followed by review of the resulting mass spectra. In general, higher fragmentor voltage helps the transmission of ions through the relatively high-pressure region between the exit of the capillary and the entrance of the skimmer[4]. At voltages of 30 V or less, very little fragmentation was observed. At voltages of 50 V or greater, excessive fragmentation occurred and there was very little signal observed at $[M + H]^+$ (m/z = 261). At voltages greater than 100 V, neither the protonated molecular ion nor the potassium adduct, $[M + K]^+$ (m/z = 299), was observed in the mass spectrum. A fragmentor voltage of 40 volts was chosen for this analysis because it allowed the detection of structurally useful fragment ions while maintaining sufficient response for the molecular ion.

For screening the samples, full scan MS data was used. Figure 3 depicts a total ion chromatogram for one of the injections of the mid-range standard, and figure 4 shows the corresponding mass spectrum for the carisoprodol peak.

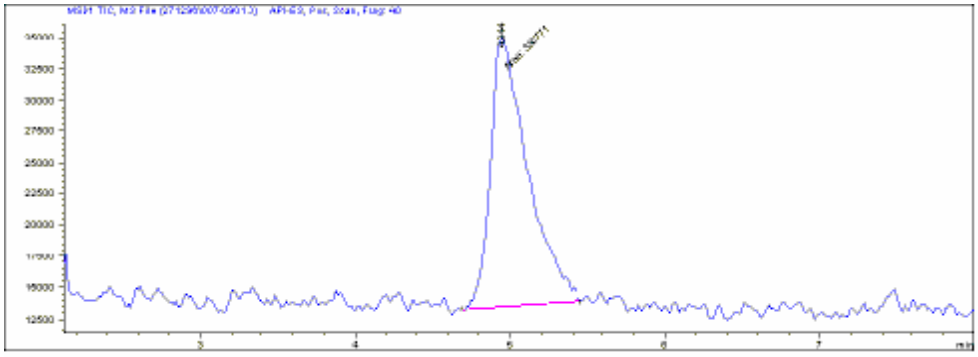

Figure 3: Full-Scan Total Ion Chromatogram for 20µg/mL Carisoprodol Standard

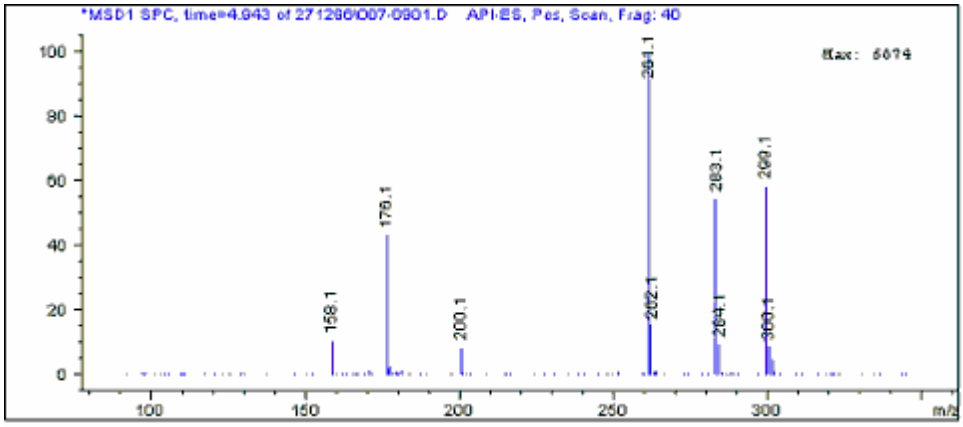

Figure 4: Mass Spectrum of 4.9 min peak in 20µg/mL Carisoprodol Standard Chromatogram

Several of the ions observed in the mass spectrum are considered structurally significant. The ion observed at m/z 261 represents the protonated molecular ion $[M + H]^+$, and the ion observed at m/z 299 represents a potassium adduct $[M + K]^+$. The fragment at m/z 200 is indicative of a loss of a carbamate ion. The likely source of the m/z 158 fragment is through a McLafferty rearrangement of the m/z 200 fragment, and subsequent loss of an isopropyl group. The fragment at m/z 176 is representative of a loss of isopropylformamide from the molecular ion. Figure 5 illustrates the proposed fragmentation pathways.

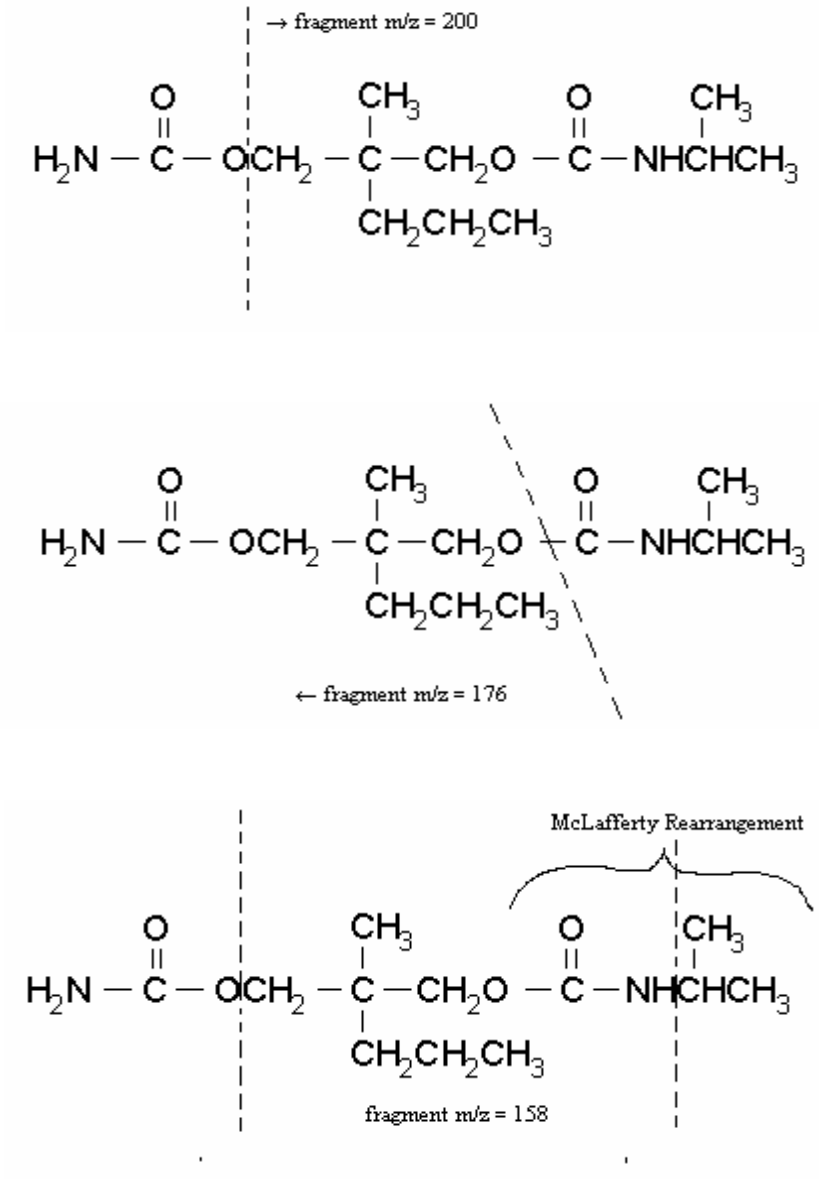

Figure 5: Proposed Fragmentation Patterns for Carisoprodol by ESI-LC-MS

For the determination of the carisoprodol content in the tablets, the SIM data was used. The protonated molecular ion (m/z = 261) was monitored in the SIM experiment. Figure 6 provides an example of a carisoprodol SIM chromatogram. The concentration of carisoprodol present in the tablets was not declared, but based on internet research, the tablets were purported to contain 350 mg carisoprodol each[1]. Five individual tablets were assayed, with results ranging from 322 mg to 329 mg carisoprodol per tablet, and a mean concentration of 325 mg carisoprodol per tablet. This range of values represents an RSD of 1.0 %. Assuming the "declared" value for the tablets is 350 mg, the average value of 325 mg/tablet translates to 93 % of label claim.

Two individual tablets were each spiked with a portion of the USP Carisoprodol reference standard. Tablet 1 was spiked with carisoprodol at a level 0f 554 mg/g, and tablet 2 at a level of 913 mg/g. Recoveries of the carisoprodol were 90 % and 95 %, respectively.

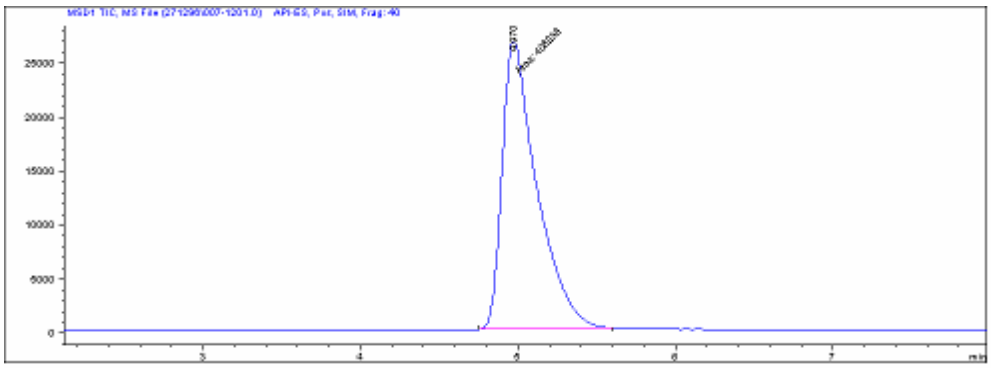

Figure 6: SIM Chromatogram for 20µg/mL Carisoprodol Standard

Conclusions

The analysis of carisoprodol tablets was performed using liquid chromatography with an electrospray interface and mass selective detection. The results obtained for five individual tablets ranged from 322 mg/tablet to 329 mg/tablet with an RSD of 1 %. The accuracy of the method was demonstrated by spike recoveries of 90 – 95 %. The on column detection limit was determined to be 6.7 picograms for carisoprodol. By using the technique discussed, finished dosage forms can be screened for the presence of carisoprodol and the carisoprodol content can accurately be determined.

Acknowledgements

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