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Analysis of magnesium from magnesium stearate in pharmaceutical tablet formulations using hydrophilic interaction liquid chromatography with nano quantity analyte detection

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ABSTRACT

This study demonstrates the use of hydrophilic interaction liquid chromatography with a nano quantity analyte detector for the retention, separation and detection of magnesium from magnesium stearate in tablet formulations for a drug product formulation blend containing a hydrochloride salt of a weakly basic compound as the active ingredient. The nano quantity analyte detector can provide direct detection of inactive excipients and inorganic salts lacking ultraviolet chromophores, as well as, all non-volatile compounds. The separation was accomplished using a SeQuant ZIC®-HILIC column and mobile phase consisting of 32.5:32.5:35 of acetone/methanol/ammonium formate buffer (150 mM, pH 4.5). Common validation parameters were evaluated to assess the method's quantitative potential for magnesium (from magnesium stearate) including: linearity, accuracy, specificity, solution stability, repeatability, and intermediate precision. Overall, the method described in this report proved to be very robust and represents a novel technique to conveniently separate and detect magnesium from magnesium stearate in pharmaceutical preparations both quickly and accurately.

1. Introduction

Excipients and other additives (inactive ingredients) are typically used in pharmaceutical drug products to enhance stability, control release, increase absorption and aid in the manufacturing process. Magnesium stearate is often added as a lubricant in capsule or tablet drug product manufacturing to prevent the excipient blend from the tablet or capsule contents from sticking to the manufacturing equipment. The strongest inter-particulate bonds are formed between clean surfaces. The mechanism by which magnesium stearate acts as a lubricant is believed to be the interruption of inter-particulate bond formation through formation of a thin hydrophobic film around particles. Such a film can often result in softening of tablets and retard release and dissolution of the active pharmaceutical ingredient. The desirable effect of lubrication and the undesirable effects of tablet softening and poor dissolution are a function of magnesium stearate concentration and its blending time. Magnesium stearate contains two equivalents of the anion stearate for each magnesium cation (see Fig. 1). Magnesium stearate exhibits very low solubility in aqueous media which adds another challenge for analysis. During the formulation development process and excipient compatibility testing, analytical methodologies are needed to quantify the drug product components to ensure mixture integrity, excipient compatibility (drug-excipient interactions) and homogeneity. Several spectrophotometric methods, such as atomic absorption [1], near-infrared spectroscopy [2], laser-induced breakdown spectroscopy [3] and energy dispersive X-ray fluorescence [4] have been employed for magnesium stearate analysis. High-performance liquid chromatography (HPLC) has also been utilized by Arai and Hosoi [5] using pre-column derivatization with 2-nitrophenylhydrazine to enable reversed-phase HPLC with UV detection. Hydrophilic interaction chromatography (HILIC) using zwitterionic stationary phases with evaporative light scattering detection (ELSD) or charged aerosol detection (CAD) and mixed-mode chromatography with CAD were shown to be excellent alternative methods for the simultaneous retention, separation and analysis of anions and cations [6–9]. A review article thoroughly discussed and compared several techniques (including HILIC) for counterion analysis [10]. Andrew Alpert first coined the term HILIC for the separation of proteins, peptides, and polar

Abbreviations: CN, condensation nucleation; CNLSD, condensation nucleation light scattering detector; ELSD, evaporative light scattering detection; HILIC, hydrophilic interaction chromatography; NQAD, nano quantity analyte detector; R^2, coefficient of determination.

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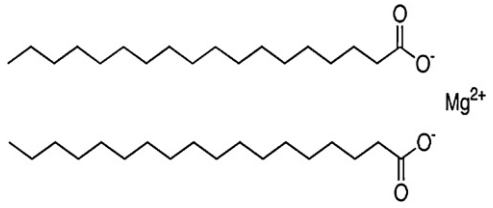

Fig. 1. Chemical structure of magnesium stearate.

molecules [11], although this mechanism had been previously established for the separation of carbohydrates [12,13]. The HILIC mode has also been successfully used for polar pharmaceutical compounds [14,15] and for enantiomeric separations [16,17].

The nano quantity analyte detector (NQAD), which was originally pioneered under the name condensation nucleation light scattering detector (CNLSD) by Allen and Koropchak [18], is a relatively new commercial detector for HPLC. The design of the NQAD is similar to that of traditional aerosol detectors, such as the ELSD, with some significant enhancements. Analogously to the ELSD, the NQAD utilizes the process of aerosol generation (by pneumatic nebulization), producing a wet aerosol of mobile phase components and analyte, which then goes through desolvation (drying) to produce, ideally, a dry aerosol of involatile analyte. As opposed to ELSD, were the light scattered by the dry particles is measured and utilized as the basis for the detector signal, the NQAD adds the intermediate step of condensation nucleation. In this step, supersaturated vapor (water in the case of the current commercial NQAD) is condensed onto the dry particles, growing them into droplets that are easily detectable by light-scattering. Since particles that are too small to be detected by regular ELSD can still be efficiently grown to droplets by condensation nucleation and detected, the sensitivity of NQAD is significantly enhanced over that of ELSD [19,20]. Other figures of merit, such as linearity, have also been reported to be improved over the ELSD [20]. Interfacing of CNLSD/NQAD with various separation systems and modes, such as HPLC (various modes), supercritical fluid chromatography, and even capillary electrophoretic techniques, has been reported for a wide variety of analyte classes [20,21].

In this report, the combination of HILIC with NQAD is used to retain, separate and detect magnesium from magnesium stearate, as well as, accurately quantitate magnesium in pharmaceutical formulation blends. Such ability makes it possible to determine the optimum blending time required to achieve the desirable lubrication effect and avoiding tablet softening.

2. Experimental

2.1. Chemicals

The organic solvents, acetone from Mallinckrodt (Paris, Kentucky), methanol from EMD (Gibbstown, NJ), ethanol from Decon Labs, Inc. (King of Prussia, PA) and acetonitrile from Fisher Scientific (Fairlawn, NJ), were all HPLC grade. Deionized water and nitrogen were from an in-house system from Eli Lilly and Company (Indianapolis, IN). Ammonium formate was obtained from Fluka Chemika (Buchs, Switzerland). Formic acid, acetic acid and trifluoroacetic acid (TFA) were acquired from Sigma–Aldrich (St. Louis, MO). Magnesium stearate was acquired from Riedel-de Haen (Hanover, Germany), Fluka Chemika, or Sigma–Aldrich. A standard stock solution of magnesium was purchased from Alltech Associates (Deerfield, IL). Magnesium chloride hexahydrate, lithium chloride, sodium acetate, potassium acetate and calcium acetate were purchased from Sigma–Aldrich (St. Louis, MO). The drug substance, a hydrochloric acid salt of a weakly basic compound, and the formulation blend were prepared at Eli Lilly and Company (Indianapolis, IN).

2.2. Equipment

Chromatographic analysis was performed on an Agilent 1100 HPLC system (Palo Alto, CA) equipped with a binary gradient pump, autosampler, a temperature-controlled column compartment and a diode array detector. The HPLC system was also integrated with a NQAD from Quant Technologies (Blaine, MN). The NQAD settings were as follows: gas flow pressure 30 psi, evaporation temperature 45 °C and a gain setting of 1. The separation was accomplished using a SeQuant ZIC®-HILIC column (250 mm × 4.6 mm, 5 μm particle size) purchased from the Nest Group, Inc. (Southborough, MA). The mobile phase for the isocratic method was 32.5:32.5:35 of acetone/methanol/ammonium formate buffer (150 mM, pH 4.5) with a flow rate of 1.0 mL/min. The injection volume was 5 μL, the column temperature was maintained at 25 °C and the autosampler temperature was set at 20 °C. The ultraviolet (UV) detector signal was also monitored using absorbance at 210 nm during development, but was not monitored in the final assay.

2.3. Standard and sample preparation

Magnesium reference solutions and standards were prepared from the Alltech magnesium standard stock solution (1002 μg/mL) in a concentration range from 5.0 μg/mL to 100.2 μg/mL with sample solvent (49:49:2, acetone/methanol/TFA) by pipetting 50, 100, 250, 500 and 1000 μL into separate 10.0 mL volumetric flasks and diluting to volume with sample solvent. The standard curve was calculated by least-squares regression analysis of peak areas versus concentration. The concentration of magnesium in the samples was determined by comparing the peak area to the standard curve. For the drug product analysis, approximately 300 mg of formulation blend was accurately weighed in vials, diluted with 5.0 mL of sample solvent (49:49:2, acetone/methanol/TFA) and sonicated for 5 min. Due to the high volatility of the sample solvent, it is very important to prevent any evaporation after standard or sample preparation.

3. Results and discussion

3.1. Method development

3.1.1. Sample solvent

Selecting the proper sample solvent can be an important criterion for optimum chromatographic performance. The sample solvent is often based on the solubility of the sample and the compatibility with the chromatographic mode of the separation. Due to the poor aqueous solubility of magnesium stearate, we investigated several solvent and solvent combinations to find adequate solubility. A minimum target concentration of 2 mg/mL was used as cut-off point (minimal solubility desired). The solubility of magnesium stearate was evaluated by visual inspection after sequentially adding small amounts of media to 10 mg of magnesium stearate followed by light agitation before the next media addition. This process was followed until the magnesium stearate was completely dissolved or reaching the 5.0 mL end point. Initially, ethanol, acetone, methanol and acetonitrile were individually tested, however in each case the 5 mL end point was reached without complete dissolution of magnesium stearate. Next, 1% TFA was added to each of the same solvents and the solubility test was repeated. Solubility was attained for methanol/1% TFA (20 mg/mL), ethanol/1% TFA (22 mg/mL), acetone/1% TFA (25 mg/mL) but not for acetonitrile/1% TFA where the 5 mL end point was reached. The solubility effect of acid was then evaluated using acetone with 0.5%, 1%, 2% and 5% of