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Determination of meglumine in pharmaceutical formulations using high performance liquid chromatography

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Received April 24, 2011, accepted June 8, 2011

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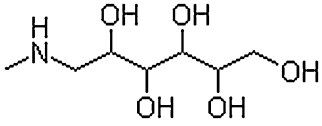
Pharmazie 66: 916–919 (2011) *doi: 10.1691/ph.2011.1059*

Four different approaches were followed for the development of a HPLC method for the determination of meglumine in solid dosage formulations: derivatization of meglumine prior to HPLC analysis, the use of an ion-pairing reagent in the mobile phase, the use of charged surface hybrid stationary phase and the use of a column designed for carbohydrate separations. The method using anionic pairing reagent in the mobile phase was shown to be suitable for the quantitative determination of meglumine in solid dosage forms. The HPLC separation was achieved on an Agilent Eclipse XDB-C_{18} column (150 mm × 4.6 mm, 3.5 μm particle size) using a mobile phase with octane-1-sulfonic acid. The method was validated and validation included the following studies: selectivity, precision (repeatability), linearity and accuracy. During validation experiments RID and DAD detectors were used.

1. Introduction

Meglumine (*N*-methyl glucamine, (2*R*,3*R*,4*R*,5*S*)-6-methylamino hexane-1,2,3,4,5-pentol), is a carbohydrate derived from sorbitol in which the hydroxyl group in position one is replaced by a methylamine group. It is widely used in pharmaceutical formulations as an excipient. The increased applicability of meglumine relates to its ability to form adducts with carboxylic acids and markedly increases their solubility in aqueous solutions due to the presence of a large number of hydroxyl groups. This property has been extensively exploited by the pharmaceutical industry due to the fact that certain drug substances are more soluble in water when taken in the form of an adduct with meglumine, without affecting the original activity of the drug. The most successful examples of such increased solubility involve the non-steroidal anti-inflammatory drugs with terminal carboxylic acid groups (Cassimiro et al. 2011).



Many examples of formulations such as oral solutions or solid dosage forms that contain a complex of meglumine with the active compound are described in the available literature, as well as in patents. Ternary systems of celecoxib, poly(vinyl pyrrolidone) and meglumine were studied to establish molecular interactions responsible for enhanced drug stability and solubility in amorphous form (Gupta and Bansal 2005). The influence of a polyhydroxy base, meglumine, as a ternary component on the complexation of a poorly water-soluble and weakly acidic anti-inflammatory drug molecule, with 2-hydroxypropyl-β-cyclodextrin was studied. It was shown that meglumine was responsible for solubility improvement *via* multiple factors (Basavaraj et al. 2006). Two patents (Veronesi 1988, Motola et al. 1991) describe the inventions related to the salts of meglumine and non-steroidal anti-inflammatory drugs. It has been found that meglumine, as the salt of ibuprofen, not only provides water solubility to the ibuprofen but also provides significant taste masking.

The importance of meglumine as an excipient is therefore obvious and the need for analytical method suitable for its quantification in pharmaceuticals is evident. While liquid chromatographic methods for separation and quantification of different carbohydrates, as well as amino sugars such as glucosamine, have been described (Shao et al. 2004), there are rather scarce data in literature describing the analysis of meglumine. Due to the absence of a chromophore, the assay of sugars and amino sugars is usually determined using high performance liquid chromatography (HPLC) with different derivatization reactions prior to the analysis (An et al. 2003). Derivatization techniques for HPLC analysis have received significant attention because they enable a highly sensitive detection of these compounds by binding a chromophore that results in products with strong UV absorption (Liang et al. 1999, Meyer et al. 2001, Aghazadeh-Habashi et al. 2002, Fischer et al. 2003, Bravo et al. 2004, Sitanggang et al. 2009). An alternative approach to derivatization and subsequent HPLC analysis is based on the use of ion-pairing reagents dissolved in the mobile phase to enable retention and provide acceptable peak shape of polar analytes (especially amines) on reversed phase columns.

The purpose of this research was to develop a suitable HPLC method for determination of meglumine in commercially available solid dosage forms and to validate the developed method. Meglumine is a small, polar molecule which has poor retention characteristics in reverse phase liquid chromatography. Furthermore, it has no chromophore which makes its detection demanding. Based on existing literature data and new stationary phases available on the market, four different approaches

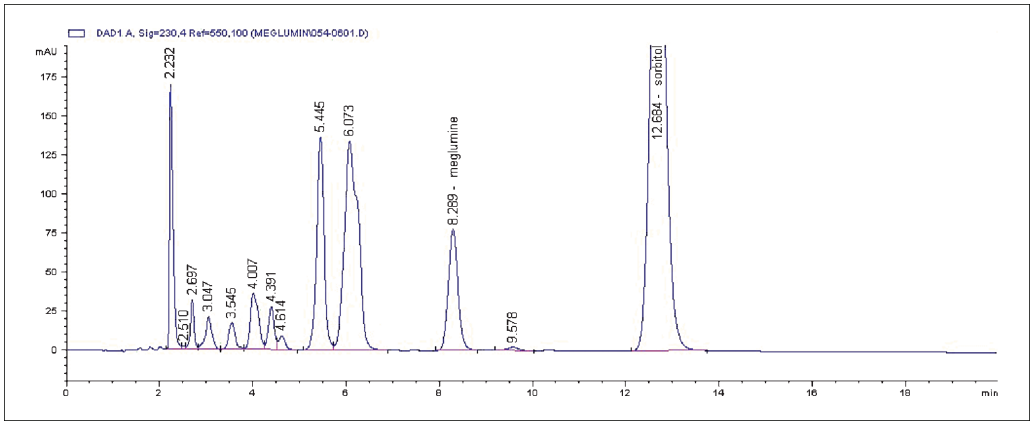


Fig. 1: Chromatogram of the derivatized *in-house* formulation (solid dosage form) coded as **A1**

were employed for the development of the HPLC method for the determination of meglumine in solid dosage formulations: derivatization of meglumine prior to the HPLC analysis, the use of an ion-pairing reagent in the mobile phase, the use of charged surface hybrid stationary phase and the use of column designed for carbohydrate separations. As a derivatization technique, we evaluated sugar benzoylation reaction based on the reaction described in the paper of Miyagi et al. (2007). A reversed phase ion-pairing HPLC method based on the chromatographic system for determination of glucosamine was adapted for meglumine separation (Way et al. 2000). The use of a new reversed stationary phase, charged surface hybrid phase, was compared to the use of octanesulfonate as an ion-pairing reagent and a component of the mobile phase. As the last chromatographic approach, we tried to use a column especially designed for the separation of carbohydrates, on which the separation of sorbitol was previously successfully experienced.

2. Investigations, results and discussion

2.1. Derivatization of meglumine

Meglumine was derivatized, according to the literature procedure (Vogel 2006), using benzoyl chloride in the presence of sodium hydroxide as a base. Benzoyl chloride was added to the aqueous solution of meglumine and sodium hydroxide. After the extraction by vortexing, organic layer was analyzed using DAD detector at 230 nm. Samples were prepared in triplicate in order to test the reproducibility of the sample preparation. Unfortunately, preparation of the derivatized meglumine was not reproducible (RSD for triplicate preparations of three meglumine concentration levels ranged from 8.5 to 28.4%). The method was shown to be suitable only for identification and not for quantification purposes (an example of chromatogram obtained after the analysis of derivatized sample is shown in Fig. 1). A plausible explanation could be the fact that the derivatization reaction is perhaps not quantitative under the mentioned conditions.

2.2. Ion-pairing HPLC method with refractive index and diode array detector

The method was defined and validation of the method included the following studies: selectivity, precision (repeatability), linearity and accuracy. Both detectors, RID and DAD, were used during validation experiments.

The method was found to be selective; separation of meglumine in *in-house* formulation (solid dosage form) coded as **A1** is presented in Fig. 2, where it can be clearly seen that meglumine peak is separated from all other components.

Precision (repeatability) was tested using in-house formulation **A1**. Samples were prepared in hexaplicate in the final concentration of meglumine of 0.24 mg/ml. The relative standard deviation of meglumine assay results obtained from six sample preparations was less than 2%.

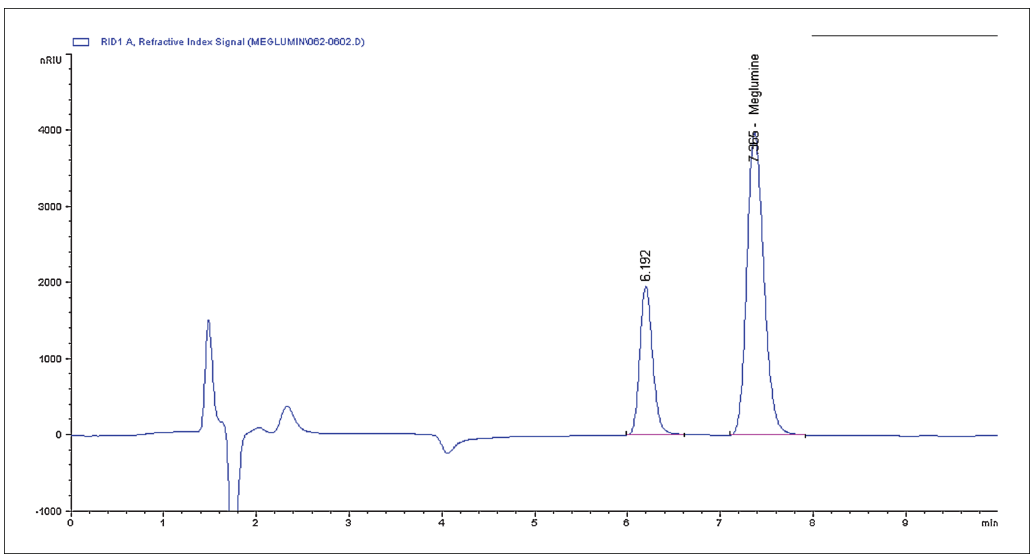


Fig. 2: Chromatogram of the *in-house* formulation (solid dosage form) coded as **A1** (RID detector)

The working concentration of meglumine in *in-house* formulation **A1** *Sample Solution* was set to 0.24 mg/ml. The linearity study for meglumine was designed to cover the range of about 50% to about 150% of the working concentration. This was equivalent to a range of about 0.12 mg/ml to 0.36 mg/ml of meglumine. A set of five meglumine standards at the following concentrations were prepared: 0.122, 0.192, 0.2442, 0.290 and 0.360 mg/ml, all injected in triplicate. The linearity study was analyzed as follows: each area response was plotted versus corresponding concentration and linear regression analysis without forcing the line through the origin was performed (correlation coefficient of the regression line was determined). The correlation coefficient was equal to 0.9996, indicating excellent linearity. The percent relative standard deviations of the peak areas of three replicate injections were found to be between 0.01% and 0.06%.

Accuracy of the method was evaluated by preparing a sample of an *in-house* formulation **A1** with the content of the meglumine at four concentration levels. Recoveries of meglumine ranged from 94.1% to 100.1%.

Although meglumine does not exhibit a characteristic UV-VIS spectrum, detection at the wavelength of 195 nm was possible. For that purpose the adjustment of the working sample concentration to 1 mg/ml was needed.

After method validation, in order to prove suitability of the method two commercially available solid dosage formulations coded as **B1** and **B2** were analyzed. Meglumine assay was determined and its content in the formulation **B1** was equal to 23.8 mg per tablet, while in **B2** formulation it was 0.61 mg per tablet.

2.3. Use of charged surface hybrid stationary phase and column designed for carbohydrate separation

Based on Charged Surface Hybrid (CSH^{TM}) Technology, this HPLC column family offers superior peak shape improvements for basic compounds when operated in acidic, low ionic strength mobile phases. Unfortunately, for meglumine peak in chromatographic setup described in the Experimental section this column did not produce acceptable peak shape. The same was noticed by using Bio Rad Aminex column for carbohydrate analysis.

Based on the results obtained, it can be seen that only the method using ion-pairing reagent in the mobile phase was acceptable for the analysis of meglumine in solid dosage forms. Although meglumine has very low response in the UV part of the spectra, detection and quantification at 195 nm was possible. By using the refractive index detector, complicated and time consuming chromophore modification of meglumine (which was shown to be irreproducible) became completely unnecessary. The use of anionic pairing reagent and separation on an ordinary C18 column was found to be superior to the use of new column with stationary phase based on Charged Surface Hybrid (CSH^{TM}) Technology and column for carbohydrate analysis. The described method is rather simple, and therefore suitable for meglumine analysis in solid dosage forms.

3. Experimental

3.1. Materials

Meglumine (99.6% purity), used in experiments as a standard, was purchased from Merck, Germany. Methanol and acetonitrile were HPLC grade, while octane-1-sulphonic acid was p.a. grade, all purchased from Merck, Germany. Potassium dihydrogen phosphate, dipotassium hydrogen phosphate, phosphoric acid, sodium hydroxide and chloroform were p.a. grade and all were purchased from Kemika, Croatia. Benzoyl chloride was obtained from Sigma-Aldrich, Germany. HPLC grade water was prepared using a Millipore Milli-Q water purification system. An in-house formulation (solid dosage form) coded as **A1** was used for the method validation (each tablet contained a declared amount of meglumine). In addition, 2 commercially available solid dosage forms containing meglumine, coded as **B1** and **B2**, were analyzed using developed method.

3.2. Instrumentation

HPLC analysis was performed using Agilent 1100 instrument equipped with DAD and RID detectors (Agilent Technologies, Santa Clara, CA, USA).

3.3. Derivatization of meglumine (Vogel 2006)

Analyses were performed using the Waters Spherisorb ODS2 column (4.6 mm × 125 mm, 3 μm) with an in-line filter. Elution was isocratic; mobile phase consisted of acetonitrile-dipotassium hydrogen phosphate buffer (25 mM, pH = 7.5) (75:25, v/v). The run time was 20 min, with a flow rate of 1.0 ml/min. The detection wavelength, injection volume and column temperature were 230 nm, 1 μl and 25 °C, respectively.

Meglumine was weighed into three 50 ml plastic cuvettes (12.8, 25.6 and 38.4 μmol), followed by the addition of an excess of the 2.5 M solution of sodium hydroxide in water (2.5 mmol) and benzoyl chloride (86.1, 172.2 and 258.3 μmol, respectively). Samples were vortexed over 2 min, followed by the addition of chloroform (10 ml). Solution was vortexed again over 2 min. After the separation of layers, 1 ml of lower layer was pipetted into the vials. Samples were prepared in triplicate.

3.4. Ion-pairing HPLC method using refractive index and diode array detector

The column used was Agilent Eclipse XDB-C_{18} column, 150 mm × 4.6 mm, 3.5 μm particle size with in-line filter. The mobile phase consisted of a mixture of buffer and methanol (buffer: 5 mM solution of octane-1-sulfonic acid sodium salt in water, adjusted to pH 2.1 with H_3PO_4), 95:5, v/v. Elution was isocratic, with the run time of 10 min. The flow rate was set to 1.0 ml/min, the injection volume was 20 μl, and the column temperature was 25 °C. Diode array detector was set to 195 nm.

The method was validated and validation included the following parameters: specificity, precision, linearity and accuracy. For the purpose of method accuracy validation, an *in-house* formulation (tablet) coded as **A1** was used (tablets contained a declared amount of meglumine). All solutions of meglumine were prepared using mobile phase as diluent.

3.5. Charged surface hybrid HPLC method using diode array detector

Separation was done on Waters XSelect CSH C18 column (3.0 mm 100 mm, 3.5 μm). The mobile phase consisted of a mixture of buffer and methanol (buffer: 10 mM solution of potassium dihydrogen phosphate, adjusted to pH 3.0 with H_3PO_4), 95:5, v/v. Elution was isocratic, with the run time of 15 min. The flow rate was set to 1.0 ml/min, the injection volume was 20 μl, and the column temperature was 25 °C. Diode array detector was set to 195 nm. Meglumine test solution (concentration of meglumine was about 1 mg/ml) was prepared using mobile phase as diluent.

3.6. Bio Rad Aminex column designed for the carbohydrate separation (for fermentation monitoring) using diode array detector

Column used was Bio Rad Aminex HPLC column for fermentation monitoring (150 mm × 7.8 mm), where successful separation of carbohydrates such as sorbitol was already performed in our laboratory. Elution was isocratic with the run time of 10 min, and mobile phase consisted of 1 mM sulphuric acid. The flow rate was set to 0.8 ml/min, the injection volume to 20 μl, and the column temperature was equal to 25 °C. Diode array detector was set to 195 nm. Meglumine test solution (concentration of meglumine was about 1 mg/ml) was prepared using water as diluent.

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