Determination of Nimesulide and Thiocolchicoside Impurities in a Topical Gel Product by UPC².

Gülçin Tok, Yelda Erdem, Ediz Yıldırım, Ali Türkyılmaz, Ümit Çifter
Research & Development Department, Sanovel Pharmaceuticals, Istanbul, Turkey

A topical pharmaceutical product consisting of Thiocolchicoside and Nimesulide was analysed using UPC² for determination of impurity content. Historically, these impurities were analysed by normal phase and reversed phase LC, especially in the case of the Thiocolchicoside impurities. UPC² offered a single platform technique instead of these two. For each drug substance, separate methods were developed and validated with reference to pharmacopoeial requirements. The sample preparation for both UPC² methods is identical and is significantly simpler than the liquid chromatographic methods which included a solid phase extraction step.

Introduction

Muscle relaxants are used alone or together with nonsteroidal anti-inflammatory drugs (NSAIDs) in treating pain [1]. Thiocolchicoside (TH) belongs to the muscle relaxant class of medications and reduces muscle spasms while Nimesulide (NIM) is an NSAID that reduces pain and inflammation. TH has never been used together with NIM in combination as a topical gel formulation for treating inflammatory pain and muscle skeletal system diseases. A literature survey revealed that several analytical methods have been described for analysis of TH as single component or in combinations with other drugs [2]. Hence, no pharmacopoeial methods or any other studies include TH and NIM together in a combination formulation.

The main challenges to the analysis of this product were the high number of impurities requiring separation, and the low amount of TH and NIM in the drug product requiring significant sample preparation prior to analysis. The sample concentration needed to consider the maximum daily dose and the quantification limits (LOQ) of TH and NIM, and therefore be high enough to obtain a detectable response. A high sample concentration may also be problematic for LC systems as it may result in a pressure increase and may also be detrimental for column lifetime.

The present study demonstrates the determination of all TH and NIM impurities using two separate validated UPC² methods with high efficiency and resolution, shorter separation times, reduced solvent consumption and sampling time, streamlined sample preparation, and lower cost of analysis per sample when compared to the LC methods.

UPC² is a powerful tool and is often described as having GC like efficiency, but with wide molecular applicability. It can reduce sample preparation and analysis time by direct injection of organic solvents/ extracts and is an orthogonal separation technique to LC which means it can provide higher confidence in identifying impurities/ degradants, and it can allow full sample characterisation and separation of analytes from matrix interferences without the need for complex sample preparation [3].

Experimental

Thiocolchicoside and its related substances were analysed using the following conditions. Columns: ACQUITY UPC² BEH (2.1 x 100 mm, 1.7 µm) column and ACQUITY UPC² BEH (2.1 x 5 mm, 1.7 µm) VanGuard pre-column were used at 35°C with gradient elution at a flow rate of 1.3 mL/min; UV detection at 258 nm; the ABPR (Active back pressure regulator) was set to 1800 psi; the injection volume was 7.5 µL and sample tray temperature was thermostated at 8°C. The carbon dioxide mobile phase was modified with a co-solvent consisted of 0.01 M ammonium acetate in methanol, 2-propanol, ammonia (25 %w/v) (920:80:12, v/v/v). The LC gradient program was set as (time (min)/% co-solvent): 0/1.0, 5/18.0, 15/20.0, 17/1.0 and 20/1.0.

Nimesulide and its related substances were analysed using the following conditions. Column: ACQUITY UPC² BEH-2EP (2.1 x 100 mm, 1.7 µm) column and ACQUITY UPC² BEH-2EP (2.1 x 5 mm, 1.7 µm) VanGuard pre-column were used at 48°C with gradient elution at a flow rate 1.0 mL/min; UV detection at 230 nm; the ABPR was set to 1500 psi; injection volume 2 µL; and sample tray temperature at 8°C. The carbon dioxide mobile phase was modified with a co-solvent consisted of methanol and absolute ethanol (1:1 v/v). The gradient program was set as (time (min)/% co-solvent): 0/0.1, 3/1.0, 10/8.0, 12/8.0, 14/28.0, 17/28.0, 18/0.1 and 20/0.1.

The sample solutions for both methods were prepared as follows. Approximately 10.0 g of topical gel (equivalent to 100 mg NIM and 25 mg TH) weighed into a 100 mL volumetric flask. 50 mL acetone was added and shaken continuously to dissolve. This solution was made to volume with acetone and sonicated for 10 minutes. The solvent was then evaporated from the solution under 300-350 mbar vacuum and the solvent free solution transferred into a 20 mL volumetric flask and made to volume with acetone. (C_{TH}:1250 µg/mL, C_{NIM}:5000 µg/mL)

Spiked test solution for TH was prepared by transferring 10.0 g topical gel into a 100 mL volumetric flask, 50 mL of acetone added and shaken to dissolve and made to volume with acetone. This solution was then sonicated for 10 min. The solvent was then

evaporated from the solution under 300-350 mbar vacuum and the solvent free solution transferred into a 20 mL volumetric flask. 0.125 mg of all known TH impurities were added to the same volumetric flask and made to volume with acetone. (CTH:1250 µg/mL, C_{NIM}:5000 µg/mL, $C_{Colchicine}$:6.25 µg/mL, C_{NDNFT}:6.25 µg/mL, $C_{Cholchicoside}$:6.25 µg/mL)

Spiked test solution for NIM was prepared by transferring 10.0 g topical gel into a 100 mL volumetric flask, 50 mL of acetone added and shaken to dissolve and made to volume with acetone. This solution was then sonicated for 10 min. The solvent was then evaporated from the solution under 300-350 mbar vacuum and the solvent free solution transferred into a 20 mL volumetric flask. 0.150 mg of all known NIM impurities were added to the same volumetric flask and made to volume with acetone. (C_{TH}:1250 µg/mL, C_{NIM}:5000 µg/mL, $C_{NIM\ imp\ A}$:7.50 µg/mL, $C_{NIM\ imp}$ B:7.50 µg/mL, $C_{NIM\ imp}$ C:7.50 µg/mL, $C_{NIM\ imp}$ D:7.50 µg/mL, $C_{NIM\ imp\ E}$:7.50 µg/mL)

A placebo solution was prepared by transferring approximately 10.0 g topical gel placebo into a 100 mL volumetric flask, 50 mL of acetone added and shaken to dissolve and made to volume with acetone. This solution was then sonicated for 10 min. The solvent from the solution was then evaporated under 300-350 mbar vacuum and the solvent free solution transferred into a 20 mL volumetric flask, again making to volume with acetone.

All solutions were filtered through 0.2 µm GHP PALL, PTFE hydrophilic or PTFE hydrophobic filters.

Results and Discussion

The method parameters given in the Experimental section were determined depending on the chemical properties of the drug substances such as their solubility, polarity, log P and pKa values. Using UPC^2 as the separation system, no high pressure issues were observed with the high concentration sample (0.5 g topical gel/1 mL diluent). The same sample preparation was used for both TH and NIM methods. Preparation of the sample solution was uncomplicated, solvent friendly and less time-consuming than the comparative LC method. Although solid phase extraction was employed with the original assay, which used a LC separation mode, the column lifetime was quite short (less than 10 injections) and repeated injections could not be obtained. Three different methods were used in the LC methods which included a reverse phase for NIM, a normal phase and a reverse phase for TH. Normal phase LC methods for TH used the following conditions. Column: Silica (4.6 x 250 mm, 5 µm) at 25°C; isocratic flow rate 1.5 mL/min; UV detection at 360 nm; injection volume 20 µL; mobile phase consisted of heptane, chloroform, methanol, acetic acid (35:5:11:1, v/v/v/v); analysis time was 60 min. Reverse phase methods for TH used the following conditions. Column: C18 (4.6 x 250 mm, 5 µm) at 25oC; isocratic flow rate 1.5 mL/min; UV detection at 290 nm; injection volume 20 µL; mobile phase consisted of mixture of 45 volumes of methanol and 55 volumes of a 2.8 g/L solution of ammonium dihydrogen phosphate adjusted to pH 7.3 with tri ethyl amine; analysis time was 60 min. LC methods for NIM used the following conditions. Column: ODS (3.9 x 150 mm , 4.0 µm) at 30°C; isocrotic flow rate 1.3 mL/min.; UV detection at 230 nm; injection volume 20 µL; mobile phase consisted of a mixture of 35 volumes of acetonitrile and 65 volumes of a 1.15 g/L solution of ammonium dihydrogen phosphate adjusted to pH 7.0 with ammonia; analysis time was 60 min. The advantages of UPC^2 methods over the comparative HPLC methods were shown in the Table 1.

Acetone was chosen as the diluent since both TH and NIM were soluble in it, miscible with water coming from the gel product and also compatibility with both CO_2 and the co-solvents selected. In the TH method, better resolution was obtained between TH and N-Deacetyl N-formyl thiocholchicoside (NDNFT) when the percentage of ammonia in the co-solvent was 1.2 %v/v. Although the resolution was increased by increasing the ammonia content, baseline noise became worse. As TH is more polar than NIM, it was more retained on column, and its retention time was correspondingly later than that of NIM.

The elution program of the NIM method started with 99.9% of CO_2 in the mobile phase because the impurities E-C-B were less polar compared to NIM itself and impurities A-D, and we aimed to retard their elution from the column. In addition, the ABPR parameter of the system was set at 1500 psi to retain them and to obtain a better resolution because of the mobile phase density decreases when the ABPR pressure decreases.

It may be seen from the presented

Table 1. Advantages of UPC2 methods over the comparative HPLC methods.

UPC^2	HPLC
Direct sampling	Solid phase extraction before sampling.
One sample preparation for two methods	Two different sample preparations for methods.
Long column life	Short column life
Reproducible injections (with low RSD%)	No sufficient number of injections
One method for TH	One reversed and one normal phase method for TH

Table 2. The results of the validation summary for TH and NIM methods.

Validation Parameters (Acceptable criteria)	TH	NIM
Accuracy		
% Recovery 80-120	100.2 – 105.5	93.9 - 106.9
Precision		
Repeatability %RSD≤5.0	0.9	1.5
Sensitivity		
LOD (signal/noise ≥ 3)	0.18 µg/mL	0.09 µg/mL
LOQ (signal/noise ≥ 10)	0.6 µg/mL	0.3 µg/mL
Linearity Range		
Main component (LOQ-%120)	0.6-7.5 µg/mL	0.3-6.0 µg/mL
Impurities (LOQ-%120)	0.6-7.5 µg/mL	0.3-9.0 µg/mL
Robustness		
The modified method with the original method difference between results should be ≤ 0.03%	Flow rate varied ± 7% Column temperature varied ± 6 % ABPR varied ± 3%	Co-solvent ratio varied ± 3% Column temperature varied ± 2% ABPR varied + 2%
Solution Stability		
Relative change should be ≤ 5.0 % between to the initial value	8 h at 8^{0}C	8 h at 8^{0}C

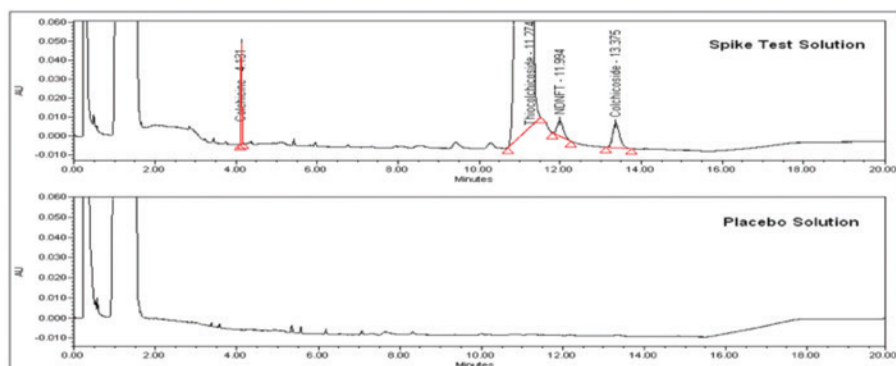

Figure 1. Analysis of Thiocolchicoside related substances by ACQUITY UPC2. Conditions noted in Experimental section.

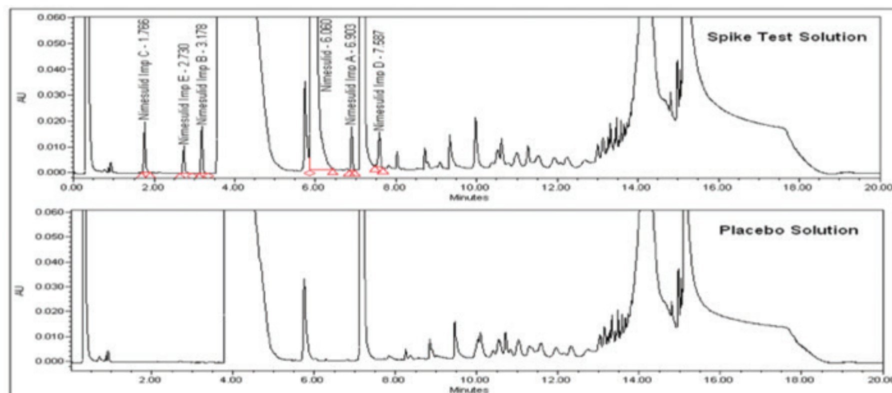

Figure 2. Analysis of Nimesulide related substances by ACQUITY UPC2. Conditions noted in Experimental section.

chromatograms that the methods developed for TH and NIM separate all known impurities and formulation excipients. Both methods were validated with reference to ICH regulations and pharmacopoeial requirements. Validation results were summarised in the Table 2.

From the validation results, the method was analyst friendly, accurate, precise, selective and reproducible. In addition to those, UPC^2 was a 'life-saver' technique for our laboratory when dealing with this complex product.

References

[1] US2011005387A1, F.Cakir, A. Turkyilmaz, L. Oner, U. Cifter, Sanovel Pharmaceuticals, (2011) http://worldwide.espacenet.com/publicationDetails/biblio?CC=US&NR=2011053877A1&KC=A1&FT=D

[2] Suraj D. Jadhav, S.R. Butle, Sachin D. Patil, P.K. Jagtap, Arabian Journal of Chemistry (2011) doi:10.1016/j.arabjc.2011.01.018

[3] Christopher J. Hudalla, Patrick D. McDonald, Waters Corporation, Milford, MA www.waters.com/upc^2

Agilent's Solutions for SFC/MSD Streamline High-Volume Analysis of Complex Compounds

Agilent Technologies, Inc recently announced that all of its LC/MS instruments now include support software for control of supercritical fluid chromatography (SFC/MS). This enhanced capability enables faster separation, orthogonal selectivity to liquid chromatography, and reduced need for organic solvents. Used together, these hardware-software solutions for SFC/MS further streamline the high-volume analysis of complex samples, making them ideal for use in a variety of industries.

Pharmaceutical, food science, lipodomic, metabolomic, environmental and petrochemical laboratories can use SFC/MS to analyze a range of compounds (e.g. chiral, achiral, polar and nonpolar), including closely related compounds in complex matrices. SFC is fully compatible with atmospheric pressure ionization mass spectrometry and hyphenation with MS detection increases peak resolving capacity, resulting in a greater SFC application range.

"The operation of SFC on Agilent's 6400 Series QQQ LC/MS and 6200/6500 TOF/Q-TOF previously required the use of two software platforms," said Lester Taylor, Agilent's director of LC/MS product marketing. "Agilent MassHunter software is now capable of controlling the Agilent 1260 Infinity Analytical SFC system. Use of a single software platform enables our customers to benefit from a fully integrated system for instrument control, data acquisition and analysis."

In addition to its analytical SFC system, Agilent is the only company offering a hybrid SFC/UHPLC system, which enables seamless switching between complementary modes in routine use and makes method development easier and faster. Both the SFC and the SFC/UHPLC system can be interfaced with Agilent LC/MS systems.

Now, reliable instrument control for method development and routine analysis is available with integrated software for SFC/MS. SFC/MS enables fast, high-resolution separation of compounds that cannot easily be separated by LC methods, with limited use of organic solvents.

For more information, please visit Agilent's online resource for extended LC systems workflow solutions. You may also visit Agilent's 2014 ASMS Media Kit to access additional product information and follow the company's full lineup of ASMS conference programming.

For further information visit www.agilent.co.uk