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Short communication

Validated high-throughput HPLC assay for nimesulide using a short monolithic column

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Abstract

High samples analysis rate is a key demand in modern pharmaceutical analysis, especially during new product development and validation of industrial-scale manufacturing process. The present study reports a validated HPLC assay for the dissolution studies of nimesulide-containing tablets (Lizepat® 100 mg/tab, Cosmopharm Ltd., Korinthos, Greece). Using a 50 mm × 4.6 mm i.d. monolithic column (Chromolith®, Merck) and acetonitrile-phosphate buffer (pH 7.0; 10 mM) (34:66, v/v) as the mobile phase, the separation cycle was completed in 60 s at a flow rate of 4.0 ml min^{-1}. The assay was validated in terms of selectivity against potential impurities of the active ingredient, detection and quantification limits, linearity, accuracy and inter-/intra-day precision. Results from the application of the HPLC method to the accelerated and long-term dissolution stability control of Lizepat® tablets (Lot 005) are reported.

Keywords: Nimesulide tablets; HPLC assay; Monolithic column; Dissolution studies

1. Introduction

A major challenge for analytical chemists employed in the pharmaceutical industry is to develop reliable and rapid methods for the control of critical quality parameters of the produced formulations. This includes not only the conformity of the finished product to established specifications (identification of active ingredient, assay, purity, dissolution, etc.) but extremely important in-process controls during production as well (blending uniformity, dosage uniformity, dissolution, etc.). Especially during validation of manufacturing processes, extensive analyses are carried out to prove the effectiveness and validity of the manufacturing process. Sampling throughput is therefore of great importance, since the results determine the following steps in the production of formulations.

HPLC is usually the predominant technique in pharmaceutical quality control, as it offers highly sensitive and selective analytical methods. Reliability of HPLC analysis can be more or less easily achieved based on the capabilities and automation of modern computer-controlled instruments and validation of both instrumentation and analytical methodologies according to international standards [1]. However, the majority of HPLC-based methodologies are time-consuming since several minutes (typically 5–30) are required for a complete separation cycle. The main reason for this is that conventional particulate-based HPLC columns (particle size typically in the range of 3–5 μm) cannot operate at elevated flow rates (>2 ml min^{-1}) due to excessive back-pressure. A very interesting alternative to particulate-based HPLC columns are monolithic columns. Monolithic columns prepared from organic and silica monomers offer very efficient separations at low back-pressure. Merck has recently commercialized silica-based reversed phase monolithic columns (Chromolith®) [2,3] suitable for HPLC applications in many analytical fields, such as food, pharmaceutical, environmental and biomedical analysis [4–7].

Recently published methods reporting the determination of nimesulide in pharmaceutical formulations are based on various techniques. HPLC procedures – although quite effective – employ particulate-based columns, thus requiring several minutes for completion of the separation–detection cycle [8–10]. Batch spectrophotometric and fluorimetric assays are laborious, involving several time-consuming steps prior to the

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final measurement [11–13]. Other techniques applied less often to the determination of nimesulide in its formulations include capillary electrophoresis (CE) [14], thin layer chromatography (TLC) [15], near-IR spectrometry [16] and adsorptive stripping voltammetry [17].

The present work reports an HPLC procedure for the high-throughput assay of nimesulide using a short monolithic column (Chromolith®, 50 mm × 4.6 mm i.d.). The ability of the column to operate at high flow rates with low back-pressure enabled the completion of the separation-detection cycle in 60 s. The assay was validated in terms of selectivity against potential impurities of the active ingredient, detection and quantification limits, linearity, accuracy and inter-/intra-day precision. Results of the application of the method to the accelerated and long-term dissolution stability control of Lizepat® tablets (Lot 005, Cosmopharm Ltd., Korinthos, Greece) are presented.

2. Experimental

2.1. Materials

HPLC grade water and acetonitrile (ACN) were used throughout this work and were both provided by Merck (Darmstadt, Germany). All other reagents were of analytical grade and were also provided by Merck, unless stated otherwise.

The mobile phase consisted of ACN-phosphate buffer (pH 7.0; 10 mM) (34:66, v/v). It was always filtered under vacuum (0.45 μm, Schleicher & Schuell) and degassed ultrasonically for 30 min prior to use.

Nimesulide micronized reference standard (lot no. 51918, assay = 99.80%), and impurities A–F (imp A: 2-phenoxy-4,6-dinitromethansulfoanilide, imp B: 2-phenoxymethansulfoanilide, imp C: 2-phenoxyaniline, imp D: 2-Phenoxy-4-nitroaniline, imp E: 2-phenoxydimethansulfoanilide and imp F: 2-phenoxy-4-nitrodimethansulfoanilide) were provided by Procos (Italy). A 1000 mg l^{-1} nimesulide standard stock solution was prepared by dissolving of an accurately weighed amount of the analyte in ACN. This solution was kept refrigerated and protected from light. Working solutions were prepared daily by appropriate dilutions of the stock in the dissolution medium. An impurities' stock solution was prepared by dissolving ca. 10 mg of each impurity in 100 ml ACN. A working impurities' solution for selectivity studies was prepared by 10-fold dilution of the impurities' stock in the dissolution medium, also containing a final concentration of 100 mg l^{-1} nimesulide.

The dissolution medium was a 50 mM phosphate buffer (pH 7.4) containing 2.5% (w/w) of polysorbate 80. The buffer was degassed under vacuum prior to the addition of the surfactant to avoid excessive foaming, and further degassed ultrasonically prior to use. When not in use, it was stored at 35 °C to minimize re-aeration.

Pharmaceutical excipients for the synthesis of the placebo mixture used in accuracy studies (sodium docusate, hydroxypropyl cellulose, lactose monohydrate, sodium starch glycolate, microcrystalline cellulose, hydrogenated vegetable oil and magnesium stearate) were obtained from domestic suppliers.

2.2. Instrumentation

An HP 1100 HPLC instrument (Hewlett Packard) was used throughout this study. It comprised a quaternary pump (G1311A), a vacuum degasser (G1322A), a column thermostat (G1316A), an autosampler (G1313A) and a DAD spectrophotometric detector (G1315A). Chromatographic parameters (peak areas, retention times, theoretical plates, etc.) were calculated via the Chem Station® software. A RP-18e monolithic column Chromolith® SpeedRod (50 mm × 4.6 mm i.d., Merck) was used for separation of the analyte.

The mobile phase was filtered using a vacuum filtration system (Schleicher and Schuell) through 0.45 μm membrane filters (RC 55, Schleicher & Schuell).

A Distek Premiere 5100 system equipped with a programmable autosampler was used for the dissolution experiments.

2.3. HPLC procedure

Twenty microliters of the samples and standards were injected in the monolithic column via the autosampler of the HPLC instrument. The flow rate was set at 4.0 ml min^{-1} ($P = 90 \pm 1$ bar) and the column temperature at 25 °C. Nimesulide was detected at 265 nm. Under the above-mentioned conditions the separation cycle was completed in 60 s. Peak areas were used for signals evaluation, while each standard was injected in triplicate and each sample in duplicate.

2.4. Dissolution of Lizepat® tablets

For each dissolution experiment, 12 nimesulide-containing tablets were weighed and introduced to the dissolution apparatus in batches of six. The temperature of the dissolution medium was kept constant at 37.0 ± 0.5 °C, while the rotation speed of the dissolution paddles was 100 rpm. The volume of the dissolution medium was 900 ml in all cases, and was degassed ultrasonically for 15 min prior to use. Two samples aliquots were withdrawn at 15 and 30 min. The samples were filtered in-line through 45 μm PTFE disc-filters. No additional pretreatment was required prior to HPLC analysis.

3. Results and discussion

3.1. Study of the chromatographic conditions

The chromatographic conditions were studied using a mixture of the active ingredient (100 mg l^{-1}) and its six potential impurities A–F (ca. 10 mg l^{-1} each). The starting conditions were 35:65 (v/v) ACN/buffer at a flow rate of 2.0 ml min^{-1} (note that the starting composition of the mobile phase was adopted from a previously used in-house HPLC assay for the determination of nimesulide using a conventional particulate-based column). Variation of the ACN fraction in the mobile phase in the range 30–40% did not affect significantly the resolution of nimesulide peak against its more closely eluted impurity (the resolution factor between nimesulide and

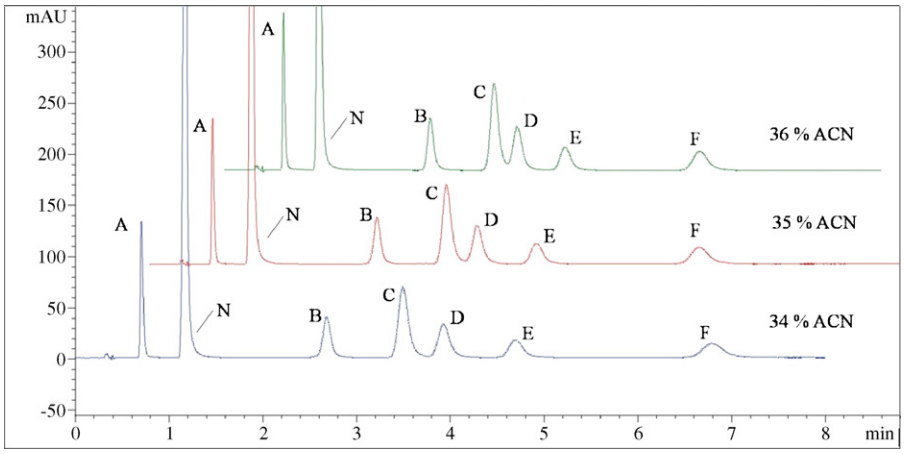

Fig. 1. Effect of the ACN fraction in the mobile phase on the separation of nimesulide (N, 100 mg l^{-1}) from its impurities (A–F, ca. 10 mg l^{-1} each). For details on the chromatographic conditions see text.

imp A was in the range of 6.5–7.5 in all cases). It affected however the separation of impurities C and D. As can be seen in Fig. 1, better separation of impurities C and D was achieved using an ACN fraction of 34% (resolution factor of 2.04), while the resolution factor was 1.76 for 35% ACN and 1.42 for 36% ACN. Separation of impurities C and D is not critical for dissolution studies of nimesulide-containing pharmaceuticals, but it proves the potentiality to expand the monolithic column-based HPLC assay to the purity control of the drug as well.

The effect of the flow rate of the mobile phase was studied in the range of 2.0–5.0 ml min^{-1}. Representative chromatograms are depicted in Fig. 2. The value of 4.0 ml min^{-1} ($P = 90 \pm 1$ bar) was selected for further studies since it allowed elution of nimesulide in 0.6 min, with an excellent base line. On the other hand, the resolution of nimesulide from its closer eluted impurity (imp A) was not affected, since the resolution factor ranged between 7.1 and 7.7 in all cases.

Based on the above-mentioned experimental findings, a system suitability test was established for the developed HPLC assay. The system suitability criterion was set at a resolution factor of not less than 2.0 between nimesulide (at the 100% level) and imp A (at the 1.0% level).

3.2. Validation of the HPLC assay

The developed HPLC assay was validated in terms of linearity, limit of detection and quantitation, within-day and day-to-day precision and accuracy.

3.2.1. Linearity, limits of detection and quantitation

The theoretically "expected" concentration of nimesulide after the dissolution experiments is 111.1 mg l^{-1} (100 mg nimesulide per tablet in 900 ml dissolution medium assuming quantitative dissolution). In order to bracket effectively the above-mentioned concentration, linearity was validated in the range of 50–150 mg l^{-1}. Six nimesulide standard solutions were used for this purpose, namely 50, 60, 75, 100, 120 and 150 mg l^{-1}. Peak area versus analyte mass concentration (in mg l^{-1}) was found to be linear within the studied range obeying the regression

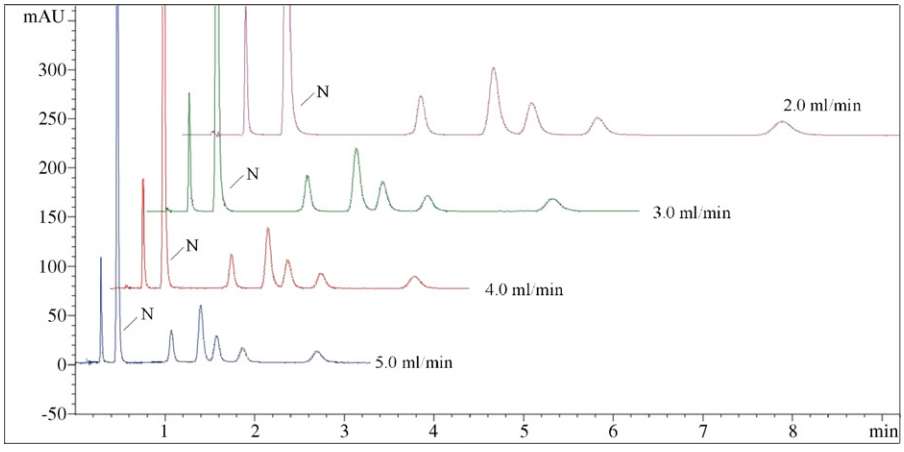

Fig. 2. Effect of the mobile phase flow rate on the separation of of nimesulide (N, 100 mg l^{-1}) from its impurities (A–F, ca. 10 mg l^{-1} each). For details on the chromatographic conditions see text.

equation:

$$A = 12.46(\pm 1.92) \times \gamma(\text{nimesulide}) + 2.54(\pm 4.11)$$

Validation of the regression line was performed by the response factor (r.f.) test [18]. The deviation of the r.f. of each point of the calibration curve must be within ±3% of the experimental slope and is given by the equation:

$$\text{r.f.} = \frac{\text{peak area} - \text{intercept}}{\gamma[\text{nimesulide}]}$$

The experimental results conform to the above-mentioned limit (±3%), since the deviations of all points of the calibration curve were in the range of −0.9 to +1.8% of the slope of the corresponding regression equation.

The detection (LOD) and quantitation limits (LOQ) of the assay were determined based on the S/N criteria. The respective values were found to be 0.050 mg l^{-1} (S/N = 3) and 0.170 mg l^{-1} (S/N = 10), respectively.

3.2.2. Within and day-to-day precision

The within-day precision of the HPLC assay was validated by calculation of the relative standard deviation of six consecutive injections of a nimesulide standard solution (100 mg l^{-1}) at the beginning, middle and end of a working day. The calculated respective R.S.D.s were 0.77, 0.51 and 0.58% (n = 6).

The day-to-day precision was validated by constructing six consecutive calibration curves (50–150 mg l^{-1} nimesulide, 6 calibration curves × 6 concentration levels). The experimental results verified the day-to-day precision of the assay. The relative standard deviation of the slopes was 1.21% (n = 6).

3.2.3. Accuracy studies

The accuracy of the method was validated by analyzing artificial samples at three nimesulide concentration levels (50, 100 and 150 mg l^{-1}). For this reason, a placebo mixture (all excipients excluding the active ingredient) was synthesized according to the manufacturing protocol of the tablets. The placebo consisted of 0.5% sodium docusate, 0.27% hydroxypropyl cellulose, 51.2% lactose monohydrate, 11.7% sodium starch glycolate, 33.3% microcrystalline cellulose, 2.7% hydrogenated vegetable oil and 0.33% magnesium stearate. Accurately weighed amounts of the placebo were dispersed in standard nimesulide solutions at the concentration levels mentioned above. The placebo concentration in the artificial samples was fixed at 500 mg l^{-1}. This level is 50% higher than the theoretically expected level in the dissolution vessels (300 mg placebo in 900 ml of dissolution medium). The artificial samples were shaken mechanically for 10 min and mixed ultrasonically for 15 min, filtered through 0.45 μm disposable syringe filters (Whatman) and injected in the HPLC system via the autosampler. Both within and day-to-day accuracies were evaluated by performing the analyses on 2 consecutive days by different analysts. The experimental results are presented in Table 1. The percent recoveries were excellent in all cases ranging between 99.05 and 100.74%. All samples were injected in triplicate.

Table 1
Accuracy of the HPLC assay

Artificial sample[a]	Placebo added (mg l^{-1})	Nimesulide added (mg l^{-1})	Recovery (±S.D.)[b] (%)
Day I/Analyst I			
S1	500	50.0	99.65 (±0.74)
S2	500	100.0	99.05 (±0.63)
S3	500	150.0	100.74 (±1.22)
Day II/Analyst II			
S4	500	50.0	100.32 (±0.84)
S5	500	100.0	100.72 (±0.76)
S6	500	150.0	99.16 (±0.93)

[a] Three replicates per sample.
[b] S.D. originates from the analysis of three samples.

Table 2
Dissolution studies of nimesulide tablets

Sample	Percent dissolution (% ± S.D.)[a]	
	15 min	30 min
0 months (production)	87.23 (±2.14)	95.46 (±2.89)
3 months (ACS)	88.42 (±2.69)	96.76 (±1.74)
3 months (LTS)	88.98 (±1.97)	96.03 (±2.35)
6 months (ACS)	86.92 (±2.22)	94.88 (±3.76)
6 months (LTS)	88.02 (±1.56)	94.57 (±1.96)
9 months (LTS)	86.34 (±3.04)	96.89 (±2.05)
12 months (LTS)	87.78 (±2.67)	95.06 (±1.63)
18 months (LTS)	89.00 (±2.88)	96.84 (±2.37)

ACS, accelerated stability; LTS, long-term stability.
[a] Mean of 12 tablets.

3.3. Application to dissolution quality and stability control

The results of the application of the assay to the dissolution quality and stability control of Lizepat® tablets (100 mg nimesulide per tablet, lot 005, Cosmopharm Ltd., Korinthos, Greece) are presented in this section. The accelerated stability (ACS) test involves the storage of the formulation at a temperature of 40.0(±2) °C and a relative humidity of 75(±5)% for 6 months, while the long-term stability (LTS) test involves storage of the formulation at a temperature of 25.0(±2) °C and a relative humidity of 60(±5)% for a period of 36 months. The dissolution results obtained during the production of the tablets correspond to "0 months". The experimental data is presented in Table 2. Note that the established specifications of the product require not less than 70% dissolution of the tablets at 15 min and not less than 85% at 30 min. From the results included in Table 2, it is clear that the formulation conforms to these specifications in all cases.

4. Conclusions

The demand for rapid, high-throughput assays in industrial pharmaceutical analysis is fulfilled by the incorporation of a short monolithic column in an HPLC system for the dissolution studies of nimesulide-containing formulations. The monolithic column enabled less-than-a-minute separation with excellent analytical features (linearity, precision, accuracy and selectivity). The rapidity of the assay is an extremely important

advantage over HPLC methods using particulate-based columns, especially during new product development and validation of manufacturing processes.

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