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Determination of Naproxen using derivatization with MSTFA in pharmaceutical preparations by GC-MS method

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ABSTRACT

This work reports the using of N-methyl-N-(trimethylsilyl) trifluoroacetamide for the derivatization of naproxen and the GC-MS method for naproxen detection in pharmaceutical preparations. The linearity of the GC-MS technique was proven over the concentration range of 0.5-12 µg/mL. There was less than 1.20 and 1.73% for the intra- and inter-day relative standard deviation, respectively. For the GC-MS technique, the limits of detection and quantification were established at 0.05 and 0.15 µg/mL. The developed technique was used to analysis tablets of a pharmaceutical preparation called Aleve. At the chosen assay conditions, tablet excipients did not cause any interference.

Keywords: Naproxen, GC-MS, Derivatization, Validation

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) such as naproxen (6-methoxy-a-methyl-2-naphthalene acetic acid) are commonly used to treat a variety of illnesses by providing mild to moderate pain relief.[1] NSAIDs, such as naproxen, are frequently used to treat persistent inflammation, pain, and fever because they can inhibit the cyclooxygenase (COX) enzymes, COX-1 and COX-2, which both produce prostaglandins. These compounds serve a number of vital purposes, including promoting inflammation, pain, and fever.[2] On the other hand, prostaglandins generated by the COX-1 enzyme can also promote blood coagulation, platelet support, and stomach protection. Therefore, NSAIDs have the potential to induce stomach ulcers and increase bleeding following surgery or trauma. Additionally, they are linked to a range of less serious side effects including nausea, vomiting, diarrhea, constipation, decreased appetite, rash, dizziness, headaches, and sleepiness, as well as potentially major adverse effects like kidney failure.[3]

Patients are exposed to anti-inflammatory medications for extended periods of time when they receive chronic treatment, like in the case of rheumatoid arthritis. Potential abuse and unintentional consumption of naproxen residues in food may put people at risk for health problems like nephrotoxicity, severe gastrointestinal lesions, allergies, and changes in renal function.[4]

Naproxen has been determined using a variety of techniques in plasma, serum, and urine. These techniques include high performance liquid chromatography (HPLC) using UV[5-11] or fluorescence detection,[12-16] liquid chromatography-tandem mass spectrometry (LC-MS/MS),[17,18] gas chromatography-mass spectrometry (GC-MS),[19,20] and gas chromatography-flame ionization detection (GC-FID).[21-23]

In the event that the laboratory does not have access to the most recent, costly technique, GC-MS is now an acceptable substitute for LC-MS/MS. High-resolution capillary GC-MS has not been employed as frequently as LC-MS/MS.[24-28]

Using LC-MS in full-scan MS mode, Sultan et al.[17] and Miksa et al.[18] presented two techniques to simultaneously measure and identify naproxen and other NSAIDs in human plasma. Though their run times were roughly 15 minutes and their LOQ values were relatively high at 2 µg/mL and 20 µg/mL, respectively, both techniques were sturdy and dependable.

There is currently no method available in the literature for determining naproxen by GC-MS in pharmaceutical formulations. Consequently, the O-silylation of naproxen's hydroxyl group is described in this work utilizing N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) as the silylating reagent. Linearity, stability, precision, accuracy, recovery, and sensitivity criteria were used to validate the devised approach in accordance with International Conference on Harmonization (ICH) guidelines.[23] Additionally, it is offered here the naproxen determination using GC-MS technique in pharmaceutical compositions. As a pharmaceutical preparation, Aleve tablets can be prepared with ease using the accurate, sensitive, and exact procedure suggested in this study.

METHODS

Chemicals

Sigma (St. Louis, MO, USA) provided the acetonitrile, naproxen sodium, and N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA). We purchased one Aleve tablet (220 mg naproxen sodium) from the pharmacy in Erzurum, Turkey.

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Instrumentation

An Agilent 6890N gas chromatography system (Agilent Technologies, Palo Alto, CA) outfitted with a 5973 series mass selective detector, 7673 series autosampler, and Agilent chemstation was used to perform the chromatographic study. For separation, an HP-5 MS column (30 m × 0.25 mm I.D., USA) with a film thickness of 0.25 µm was employed. Helium was employed as the carrier gas, and splitless injection was performed, with a flow rate of 1 mL/min. One µL was the injector volume. The MS detector's settings included an electron energy of 70 eV, a solvent delay of two minutes, and a transfer line temperature of 280 °C.

Preparation of the standard and quality control solutions

Naproxen stock solution was made with acetonitrile at a concentration of 100 µg/mL and refrigerated at -20 °C. For GC-MS, standard solutions of 0.5-12 µg/mL were produced. Aliquots of the standard working naproxen solution were added to the quality control (QC) solutions until the final concentrations for GC-MS were 3, 5, and 9 µg/mL.

Procedure for pharmaceutical preparations

The mass of the Aleve (220 mg naproxen tablet, which contained naproxen and a few excipients) capsules was used to compute the average capsule mass. Following its fine grinding and homogenization, a quantity of the powder was precisely weighed, put into a 10 mL brown measuring flask, and diluted with acetonitrile to the appropriate concentration. To speed up the dissolving process, the mixture was sonicated for at least ten minutes before being filtered through a Whatman 42 paper. To ensure that the final solution's naproxen concentration was within the working range, a suitable volume of filtrate was further diluted with acetonitrile before being subjected to GC-MS analysis.

RESULTS

Method development and optimization

Since naproxen is a polar molecule, separation was achieved using a capillary column covered with 95% dimethylpolysiloxane and 5% phenyl. The temperatures of the injection port and detector were set at 250 °C and 290 °C, respectively, during the development of the GC-MS technique. In order to determine the ideal temperature program, several temperature programs were examined. The first temperature was 150 °C, which was held for one minute; it was then raised to 180 °C at 40 °C/min and maintained for another minute; ultimately, it was raised to 300 °C at 30 °C/min with a final hold of 1.5 minutes. In split-less mode, the injector volume was one microliter.

An efficient trimethylsilyl (TMS) donor is MSTFA. MSTFA is used to prepare volatile and thermally stable derivatives for GC-MS by reacting with a TMS group to substitute labile hydrogens on a variety of polar molecules.[24,25] Naproxen was derivatized using MSTFA in order to improve the performance of the gas chromatographic separation (Fig. 1). The appropriate silyl (-O-TMS) groups were formed by converting the hydroxy (-OH) groups. The chemical was examined after the ideal reaction conditions were determined.

Investigations were conducted into how temperature and time affected the reaction. Consequently, acetonitrile was used to dissolve naproxen. After adding 100 µL of 2.0 µg/mL naproxen solution and 100 µL of MSTFA solution, the mixture was allowed to react for five, ten, and twenty minutes at room temperature, 50, and 75 °C. The GC-MS technique was used to quantify the final samples. Following a 10-minute standing period at room temperature, the maximum peak areas were measured.

Validation of the method

Specificity

Interferences between naproxen and the excipients were observed to examine the specificity of the two approaches. For the quantitative measurement in GC-MS, electron impact mode with selected ion monitoring (SIM) was utilized (m/z 185 for naproxen-TMS). Fig. 1 displays the mass spectrum following the derivatization of naproxen with MSTFA. Naproxen-TMS retention time for GC-MS was around 6.4 with a well-defined peak (Fig. 2).

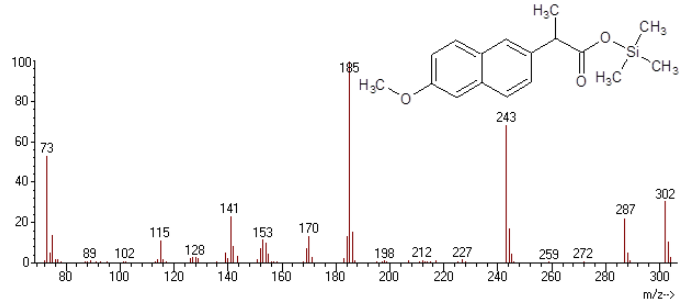

Fig. 1. MS spectrum of naproxen-TMS.

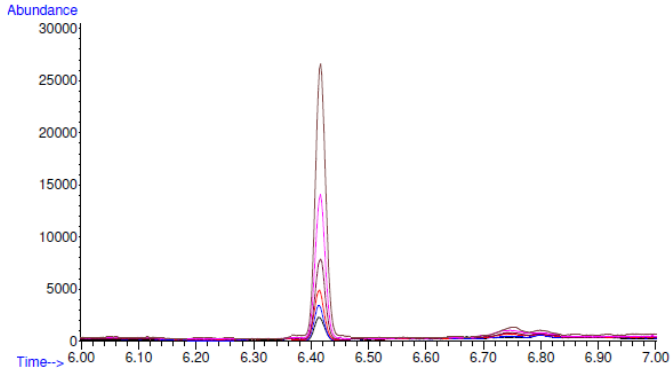

Fig. 2. GC-MS chromatogram of naproxen-TMS (0.5, 1, 2, 4, 8 and 12 µg/mL).

Linearity

The GC-MS technique revealed that the linearity range of naproxen solution was 0.5-12 µg/mL. Their correlation coefficient was used to assess the calibration curve that was built. The linearity of the procedure was shown by the calibration equation derived from six repeat trials. Table 1 provided the slope and intercept standard deviations for the calibration curves.

Table 1. Linearity of naproxen

Parameter	GC-MS
Linearity (µg/mL)	0.5-12
Regression equation[a]	y=174.4x+5.19
Standard deviation of slope	$2.58x10^{-3}$
Standard deviation of intercept	3.42
Correlation coefficient	0.9991
Standard deviation of correlation coefficient	$1.25x10^{-3}$
Limit of detection (µg/mL)	0.05
Limit of quantification (µg/mL)	0.15

[a]Based on six calibration curves, y=peak area, x=concentration of naproxen

Accuracy and precision

Repeatability (intra-day) and intermediate precision (inter-day) were used to calculate the GC-MS method's precision.[25] The study assessed repeatability by subjecting quality control samples to six daily analyses at three distinct doses. The same samples were analyzed twice a day for three days to assess the intermediate precision. The % relative error was used to evaluate the analytical method's accuracy. Relative errors were ≤4.00 % and intra- and inter-day RSD values were ≤1.73% for all concentrations examined. Table 2 presented the findings.

Limits of detection (LOD) and quantification (LOQ)

The lowest concentration of the analyte that the technique can detect is known as the LOD. The lowest measurable concentration is known as the LOQ. LOD and LOQ were determined by taking the signal-to-noise ratios of 3:1 and 10:1, respectively. For GC-MS, the LOD and LOQ were 0.05 and 0.15 µg/mL, respectively.

Stability

Standard solutions were made independently at concentrations spanning the low, middle, and higher ranges of calibration curves for various temperatures and timeframes to assess the stability of naproxen. For 24 and 48 hours, these solutions were kept at ambient temperature, chilled (4 °C), and frozen (-20 °C). For room temperature, 4 and 20 °C refrigeration temperatures, the accuracy of naproxen stability was found to be 99.8, 98.5, and 99.5%, respectively. These fall between 90 and 110 percent of what is acceptable. Table 3 presented the findings.

Recovery

Studies on recovery achieved by spiking various amounts of pure drug within the analytical concentration range of the suggested approach in tablet samples that had already been preanalyzed. Using the aforesaid procedure, the additional dosages of each medicine were estimated. The findings of the recovery experiments were deemed adequate and are displayed in Table 4.

Table 2. Precision and accuracy of naproxen

	Intra-day			Inter-day		
Added (µg/mL)	Found ± SD[a] (µg/mL)	Precision % RSD[b]	Accuracy[c]	Found ± SD[a] (µg/mL)	Precision % RSD[b]	Accuracy[c]
3	3.08 ± 0.037	1.20	2.67	3.12 ± 0.054	1.73	4.00
5	5.02 ± 0.069	1.37	0.40	4.98 ± 0.034	0.68	-0.40
9	9.03 ± 0.143	1.58	0.33	9.01 ± 0.034	0.38	0.11

SD[a]: Standard deviation of six replicate determinations, RSD[b]: Relative standard deviation, Accuracy[c]: % relative error: (found-added)/addedx100

Table 3. Stability of naproxen in solution (n=6)

	Intra-day			Inter-day	
Conc. (µg/mL)	Room temperature 24 h	Refrigeratory 4 °C, 24 h	Frozen -20 °C, 24 h	Refrigeratory 4 °C, 48 h	Frozen -20 °C, 48 h
0.5	99.4 ± 3.12	97.7 ± 4.97	99.6 ± 3.78	99.3 ± 2.16	99.1 ± 3.26
6	99.7 ± 2.08	98.6 ± 3.06	100.7 ± 3.15	99.5 ± 1.41	99.8 ± 2.19
12	100.6 ± 1.89	102.1 ± 3.54	101.8 ± 2.76	100.4 ± 2.56	100.6 ± 2.39

Table 4. Recovery of naproxen in pharmaceutical preparation

		Intra-day		Inter-day	
Pharmaceutical preparation	**Added (µg/mL)**	**Found ± SD[a] (µg/mL)**	**% Recovery % RSD[b]**	**Found ± SD[a] (µg/mL)**	**% Recovery % RSD[b]**
Aleve tablet (2 µg/mL)	4	4.11 ± 0.049	102.8 (1.19)	4.16 ± 0.093	104.0 (2.24)
	7	7.04 ± 0.079	100.6 (1.12)	7.09 ± 0.155	101.3 (2.19)
	10	10.17 ± 0.078	101.7 (0.77)	10.22 ± 0.129	102.2 (1.26)

SD[a]: Standard deviation of six replicate determinations, RSD[b]: Relative standard deviation

Comparison of the methods

Nowadays, as analytical procedures for both quantitative and qualitative analysis, GC-MS and HPLC methods are significant and often utilized. High-resolution capillary GC-MS has superior precision and accuracy with high sensitivity and strong resolving power compared to HPLC.[26]

The sensitivity of the GC-MS technique is insufficient to determine naproxen. MSTFA is utilized to boost sensitivity as a result. The derivatization procedure in this study is intended to increase sensitivity, which has allowed for the option of operating at low concentrations.

This method's sensitivity was determined to be sufficient for pharmacokinetic research when used with plasma samples. Compared to the reported approach, the current method has the following advantages.[17, 18] The current method's limit of quantification (LOQ) was 0.15 µg/mL, while the reported methods' LOQs were 2.0 µg/mL and 20 µg/mL. The naproxen calibration curve was linear over the 0.25-5.0 µg/mL concentration range, which is on par with or better than that found in earlier studies.[5-8,12-15,17,20–22]

This study has a high naproxen recovery rate.[12, 13, 18] Another benefit is the short retention duration.[5, 9, 12, 14, 17, 18, 20]

DISCUSSION

This paper presents the development and validation of a novel chromatographic method for routine measurement of naproxen in pharmaceutical formulations. Proper linearity range, accuracy, precision, LOD, and LOQ are appropriate for naproxen quantification in pharmaceutical compositions. A significant number of samples can be analyzed quickly because of the 7-minute chromatographic run time. As a result, the technique can also be used to analyze samples during expedited stability investigations as well as regular formulation and raw material analyses.

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