High-Performance Liquid Chromatographic Method Development and Validation for the Simultaneous Quantitation of Naproxen Sodium and Pseudoephedrine Hydrochloride Impurities

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Abstract

A reversed-phase high-performance liquid chromatographic procedure for the simultaneous determination of impurities associated with pseudoephedrine hydrochloride (PSEH) and naproxen sodium (NapNa) is developed and validated. The method is developed using a Waters Spherisorb cyano column (5 µm, 250 × 4.6 mm). An isocratic elution in a water–acetonitrile–methanol–triethylamine mixture (850:75:75:5) is adjusted to a pH of 3.7 ± 0.02 with formic acid as the mobile phase. The UV detection was set at 260 nm, and the wavelength was switched to 235 nm before the elution of the last component, 2-ethyl-6-methoxy-naphthalene (EMN). The method is shown to be linear at a concentration range of 0.24 to 1.92 µg/mL for benzaldehyde, benzoic acid, and 2-(methylamino)-propiophenone hydrochloride, which are known impurities of PSEH. The NapNa impurities, 2-(6'-hydroxy-2'-naphthyl) propionic acid, 2-hydroxy-6-methoxy-naphthalene, 1-(6'-methoxy-2'-naphthyl) ethanol, 2-acetyl-6-methoxy-naphthalene, and EMN are also demonstrated to be linear at a concentration range of 0.44 to 3.52 µg/mL. Under the chromatographic conditions of the method, all impurities are resolved from the active components.

Introduction

Pseudoephedrine hydrochloride (PSEH), also known as {(+)-threo-α-[1-methylamino) ethyl] benzyl alcohol} hydrochloride, is a useful bronchodilator and nasal decongestant. Clinically, it shrinks swollen mucosa membranes, increases nasal airway passages, reduces nasal congestion, and diminishes tissue hyperemia (1). Naproxen sodium (NapNa), also known as (2-(6-methoxy-2-naphthyl) propionic acid, is a nonsteroidal compound that has anti-inflammatory, analgesic, and antipyretic activities with a mean plasma half-life of 14 h (2,3).

A literature search revealed that a number of high-performance liquid chromatographic (HPLC) methods have been developed to determine NapNa degradation products in dosage forms or biological materials (4,5). No HPLC method has been reported for

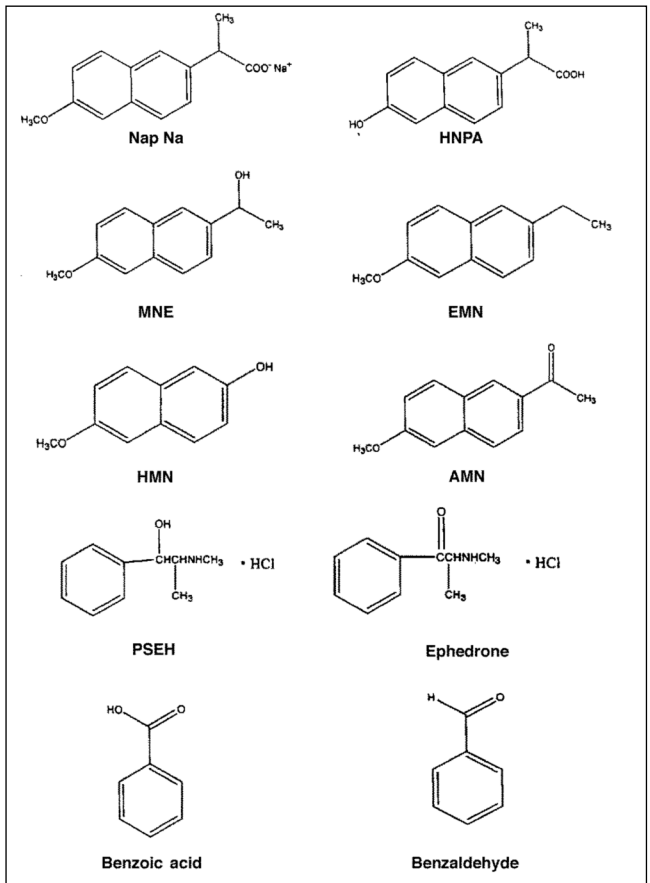

Figure 1. Structures of NapNa, PSEH, and other related substances.

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the simultaneous determination of NapNa, PSEH, and their impurities. This work was performed in order to develop an HPLC method that would allow for the separation, detection, identification, and quantitation of all impurities (Figure 1) associated with both NapNa and PSEH in a single chromatogram. The method was validated in accordance with ICH guidelines on the validation of analytical procedures (6).

Table I. System Precision Parameters

Component	%Coefficient of variation	Tailing factor	Resolution	Relative retention time (min)
PSEH	1.3	1.3	–	0.39
Ephedrone	1.5	1.3	3.5	0.43
Benzoic acid	2.9	1.2	3.6	0.48
Benzaldehyde	0.9	1.1	4.8	0.55
HNPA	1.2	1.2	3.6	0.61
HMN	1.4	1.2	9.1	0.82
MNE	2.2	1.1	5.1	1.00
NapNa	0.7	1.4	3.5	1.11
AMN	0.8	1.2	8.0	1.50
EMN	3.5	1.1	15.4	2.86

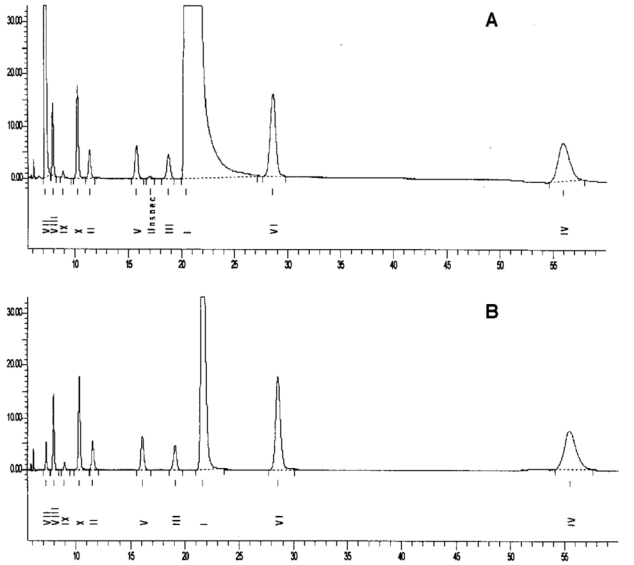

Figure 2. Representative chromatograms of (A) a spiked sample of 0.1% degradation products in the presence of NapNa at 2.2 mg/mL and pseudoephedrine HCl at 1.2 mg/mL and (B) a working degradation standard solution of 0.1% degradation products of NapNa at 44 µg/mL and pseudoephedrine HCl at 24 µg/mL. NapNa (I), HNPA (II), MNE (III), EMN (IV), HMN (V), AMN (VI), PSEH (VII), phedrone (VIII), benzoic acid (IX), and benzaldehyde (X).

Experimental

Apparatus

Liquid chromatograph

The liquid chromatographs used in this study were a PerkinElmer system (Norwalk, CT) and an Agilent Technologies Series 1100 system (Wilmington, DE). The PerkinElmer system consisted of a Series 200 IC pump, a Series 200 autosampler equipped with a 150-µL loop and a built-in refrigeration unit, a Series 235C diode-array detector with a 600 Series Link, and a Laserjet 5 printer. The Agilent Technologies Series 1100 system included a built-in refrigerated autosampler.

Column

The column used was a Waters (Milford, MA) Spherisorb Cyano (250- × 4.6-mm i.d., 5-µm particle).

Mobile phase

The mobile phase was a mixture of water, acetonitrile, methanol, and triethylamine (850:75:75:5) that was pH adjusted to 3.7 ± 0.02 with formic acid and filtered through a 0.45-µm nylon filter membrane. The flow rate was 0.5 mL/min, and the column temperature was set at 35°C.

Chemicals

HPLC-grade acetonitrile and methanol were purchased from VWR Scientific Products (South Plainfield, NJ) and used without further purification. Triethylamine and formic acid were purchased from Fisher Scientific (Springfield, NJ). Water was purified using a Milli-Q filter system (Millipore, Milford, MA). Benzoic acid, benzaldehyde, and 2-(methylamino)-propiophenone hydrochloride (ephedrone) were purchased from Sigma (St. Louis, MO). NapNa and PSEH standards were purchased from USPC, Inc. (Rockville, MD). Roche Diagnostics GmbH (Mannheim, Germany) kindly supplied the remaining impurity standards 2-(6'-hydroxy-2'-naphthyl) propionic acid (HNPA), 2-hydroxy-6-methoxy-naphthalene (HMN), 1-(6'-methoxy-2'-naph-

Table II. Limit of Quantitation

Component	Concentration (µg/mL)	Peak area responses						%Coefficient of variation
		Injection 1	Injection 2	Injection 3	Injection 4	Injection 5	Mean	
Benzoic acid	0.24	3345	3326	3467	2945	3122	3241	6.4
Benzaldehyde	0.24	37599	37133	37598	36389	37243	37192	1.3
Ephedrone	0.24	24856	25159	24902	24660	25141	24944	0.8
HMN	0.44	22175	23539	23000	22883	23893	23098	2.9
AMN	0.44	107655	103418	101933	103656	100846	103502	2.5
EMN	0.44	100040	109286	104304	86129	100081	99968	8.6
MNE	0.44	21530	20496	21626	19293	20208	20631	4.7
HNPA	0.44	15408	15014	15544	15360	15394	15344	1.3

thyl) ethanol (MNE), 2-acetyl-6-methoxy-naphthalene (AMN), and 2-ethyl-6-methoxy-naphthalene (EMN).

Analytical sample

The Bayer Consumer Care Formulation Development Laboratory (Morristown, NJ) provided the analytical sample, which was formulated to contain 220 mg of NapNa, 120 mg PSEH, and pharmaceutical excipients for each tablet.

Table III. Recovery Results for Ephedrone

		Nominal percent of 0.1% PSEH label strength				
		20	40	80	100	160
	Amount added (µg)	25.20	50.40	100.80	126.00	201.60
	%Recovery	108.4	104.9	104.4	100.7	95.6
	%Recovery	109.8	102.2	101.6	103.1	95.8
	%Recovery	107.3	101.0	104.9	101.6	94.6
%Mean recovery	Overall = 102.4	108.5	102.7	103.6	101.8	95.3
%Relative standard deviation	Overall = 4.6	1.2	1.9	1.7	1.2	0.7

Table IV. Recovery Results for Benzoic Acid

		Nominal percent of 0.1% PSEH label strength				
		20	40	80	100	160
	Amount added (µg)	24.64	49.28	98.56	123.20	197.12
	%Recovery	103.9	101.3	101.4	100.6	100.3
	%Recovery	110.1	103.8	99.3	97.7	99.9
	%Recovery	111.1	106.5	102.2	100.7	97.5
%Mean recovery	Overall = 102.4	108.4	103.9	100.9	99.7	99.2
%Relative standard deviation	Overall = 3.7	3.6	2.5	1.5	1.7	1.5

Table V. Recovery Results for Benzaldehyde

		Nominal percent of 0.1% PSEH label strength				
		20	40	80	100	160
	Amount added (µg)	24.12	48.24	96.48	120.60	192.96
	%Recovery	98.7	98.4	98.1	97.3	102.8
	%Recovery	98.2	100.0	96.8	96.7	99.3
	%Recovery	96.7	96.2	99.5	96.1	99.3
%Mean recovery	Overall = 98.3	97.9	98.2	98.1	96.7	100.5
%Relative standard deviation	Overall = 1.4	1.1	1.9	1.4	0.6	2.0

Table VI. Recovery Results for HNPA

		Nominal percent of 0.1% NapNa label strength				
		20	40	80	100	160
	Amount added (µg)	42.64	85.28	170.55	213.19	341.11
	%Recovery	102.7	102.7	99.7	100.5	105.0
	%Recovery	107.3	105.1	100.0	101.7	101.3
	%Recovery	102.2	99.5	101.2	100.4	101.0
%Mean recovery	Overall = 102.0	104.1	102.4	100.3	100.9	102.4
%Relative standard deviation	Overall = 1.5	2.7	2.7	0.8	0.7	2.2

Standard preparation

Stock impurity solution I

Approximately 8.8 mg of HNPA, HMN, MNE, AMN, and EMN (accurately weighed) were transferred into a 200-mL volumetric flask, and then approximately 100 mL of methanol was added. The mixture was sonicated to dissolve and diluted to volume with methanol and then mixed well.

Stock impurity solution II

One vial containing 1 mL of ephedrone (1.26 mg/mL in methanol) was quantitatively transferred into a 50-mL volumetric flask, diluted to volume with water, and mixed well.

Stock impurity solution III

Approximately 24.0 mg of benzoic acid and 24.0 mg benzaldehyde (accurately weighed) were transferred into a 1000-mL volumetric flask, and then approximately 50 mL of methanol was added. The mixture was sonicated to dissolve, diluted to volume with water, and mixed well.

Stock NapNa and PSEH standard solution

This stock solution was prepared by accurately weighing and transferring approximately 44.0 mg of NapNa and 24.0 mg PSEH into the same 100-mL volumetric flask. Approximately 60 mL of water was added and sonicated to dissolve, diluted to volume with water, and mixed well.

Working impurity solution

This solution was prepared by accurately transferring 10.0 mL of the stock NapNa and PSEH standard solution and 5.0 mL of stock impurity solutions I, II, and III into a 100-mL volumetric flask. The solution was then diluted to volume with water and mixed well. The final concentration of all the impurities represented 0.1% of the actives.

Preparation of samples

Assay

Twenty tablets were finely ground in a mechanical grinder, and a weighed portion equivalent to 220 mg NapNa and 120 mg PSEH was transferred into a 100-mL volumetric flask. Approximately 60 mL of warm water (40°C–45°C) was added to the flask, and it was shaken mechanically for 30 min and sonicated for 10 min. The sample was diluted to volume with water and mixed well. Using glass centrifuge tubes, a portion of it was centrifuged at 4000 rpm for 30 min. Using glass pipettes, an aliquot of the solution was directly transferred into an autosampler vial.

Recovery

Aliquots of stock impurity solutions I, II, and III were pipetted into different 100-mL volumetric flasks containing the same amount of sample as in the assay sample preparation. The samples were then prepared according to the assay procedure. The final concentrations of benzoic acid, ephedrone, and benzaldehyde were in the range of 0.24 to 1.92 µg/mL. The final concentration for all other impurities were in the range of 0.44 to 3.52 µg/mL. These concentrations represented 0.02% to 0.16% of the actives in the formulation.

Table VII. Recovery Results for HMN

		Nominal percent of 0.1% NapNa label strength				
		20	40	80	100	160
	Amount added (µg)	43.85	87.71	175.41	219.27	350.82
	%Recovery	98.5	99.6	102.2	101.1	103.6
	%Recovery	104.3	100.0	101.4	102.4	103.6
	%Recovery	102.8	98.0	101.6	101.7	103.3
%Mean recovery	Overall = 101.6	101.9	99.2	101.7	101.8	103.5
%Relative standard deviation	Overall = 1.5	3.0	1.1	0.4	0.7	0.1

Table VIII. Recovery Results for MNE

		Nominal percent of 0.1% NapNa label strength				
		20	40	80	100	160
	Amount added (µg)	44.66	89.33	178.66	223.32	357.32
	%Recovery	104.5	104.0	103.1	101.3	102.0
	%Recovery	105.7	101.9	100.5	103.2	102.4
	%Recovery	104.3	101.5	102.8	101.1	102.3
%Mean recovery	Overall = 102.7	104.8	102.5	102.1	101.9	102.2
%Relative standard deviation	Overall = 1.2	0.7	1.3	1.4	1.2	0.2

Table IX. Recovery Results for AMN

		Nominal percent of 0.1% NapNa label strength				
		20	40	80	100	160
	Amount added (µg)	43.62	87.23	174.47	218.08	348.93
	%Recovery	93.5	102.0	101.6	102.4	101.7
	%Recovery	96.4	102.7	101.4	102.7	102.0
	%Recovery	96.4	97.9	102.3	101.6	102.0
%Mean recovery	Overall = 100.5	95.5	100.9	101.8	102.2	101.9
%Relative standard deviation	Overall = 2.8	1.8	2.6	0.5	0.5	0.2

Table X. Recovery Results for EMN

		Nominal percent of 0.1% NapNa label strength				
		20	40	80	100	160
	Amount added (µg)	44.98	89.96	179.91	224.89	359.83
	%Recovery	99.4	99.9	92.6	111.4	91.0
	%Recovery	106.8	99.0	100.6	103.8	100.0
	%Recovery	88.4	85.3	97.5	104.8	95.6
%Mean recovery	Overall = 98.4	98.2	94.7	96.9	106.7	95.5
%Relative standard deviation	Overall = 4.9	9.4	8.6	4.1	3.9	4.7

Chromatographic conditions

The UV detector was programmed to switch wavelengths from 260 nm to 235 nm at 5 min before the peak of EMN. The injected volume was 20 µL. The samples were cooled in the autosampler to 4°C before injection. The samples were cooled in order to reduce any degradation that might occur during the entire analysis time.

System suitability tests

The system suitability was evaluated by making five replicate injections of the working impurity solution. The system was deemed suitable for use if the coefficient of variation for all the components was less than or equal to 5.0%, the tailing factor was less than or equal to 1.5%, and the resolution was 2.0% or higher.

Results and Discussion

Precision

The system precision was determined by chromatographing five injections of the working impurity solution and calculating the coefficient of variation of the peak area response, the tailing factor, and the resolution. The range for the coefficient of variation was from 0.7 to 3.5. The tailing factor was less than 1.5 and the resolution was greater than 2.0 for all the components (Table I, Figure 2B).

Limit of quantitation

An authentic sample containing an equivalent amount of 220 mg NapNa and 120 mg PSEH was spiked with all of the three stock impurity solutions. The benzoic acid response was used to estimate the spiking concentration that would result in a signal-to-noise ratio of 7:1 to 10:1. A concentration of 0.24 µg/mL representing 0.02% of the active (PSEH) resulted in an approximate signal-to-noise ratio of 7:1. Five consecutive injections of the spiked sample solution containing all of the impurities exhibited a reproducibility ranging from 0.8% to 8.6% of the relative standard deviation (Table II). The limit of detection was estimated to be 0.01% of the actives representing a signal-to-noise ratio of 3.5:1 for benzoic acid.

Range of linearity

The linearity of peak area responses versus con-

Table XI. Label-to-Label Reproducibility

	0.1% of active label strength												
	Lab 1						Lab 2						
Component	Lot 1-1	Lot 1-2	Lot 2-1	Lot 2-2	Lot 3-1	Lot 3-2	Lot 1-1	Lot 1-2	Lot 2-1	Lot 2-2	Lot 3-1	Lot 3-2	P-value of F-test in an ANOVA table*
Benzoic acid	96	96	93	90	94	86	96	94	99	98	97	95	0.4806
Benzaldehyde	96	95	93	92	88	88	94	100	94	98	94	97	0.7113
Ephedrone	94	96	97	95	95	94	99	101	98	99	99	98	0.6575
HMN	101	102	103	100	101	100	102	103	101	102	100	100	0.2113
AMN	102	104	105	101	102	103	105	109	107	107	115	102	0.9717
EMN	100	101	94	102	102	92	94	102	93	93	99	99	0.9469
MNE	110	103	107	103	101	102	101	102	101	101	97	100	0.5534
HNPA	100	101	100	99	99	98	100	101	100	100	96	98	0.1762

* Because the P-value of the F-test is ≥ 0.05 in an ANOVA analysis, there is not a statistically significant difference between Lab 1 and Lab 2 at the 95% confidence level.

Table XII. Stability of Standard and Sample Solutions

	%Recovered	
Component	Standard	Sample
Benzoic acid	95.9	99.9
Benzaldehyde	97.3	98.2
Ephedrone	96.9	98.3
HMN	97.9	100.1
AMN	96.6	98.7
EMN	100.6	100.2
MNE	96.1	99.6
HNPA	97.7	97.0

centrations was studied from approximately 0.24 to 1.92 µg/mL for benzoic acid, benzaldehyde, and ephedrone (representing 0.02–0.16% of the label strength of the active, which is PSEH in the formulation) and 0.44 to 3.52 µg/mL for all other impurities (also representing 0.02–0.16% of the label strength of the second active, NapNa). The data were subjected to statistical analysis using a linear-regression least-squares method. The range for the coefficient of variation was from 0.99526 to 0.99991. Because the method was a limit test for impurities, we were not concerned that some of the origins were not included within the 95% confidence limits. These points were acceptable with regards to the use of a single-point calibration.

Recovery

The recoveries of NapNa and PSEH impurities were assessed by spiking a placebo sample containing 220 mg NapNa and 120 mg PSEH with stock solutions of the impurities' standards. The spiking was done at five levels and in triplicates spanning 0.02–0.16% of the actives. The range of the overall recovery and coefficient of variation for all of the impurities was 98.3–102.7% and 1.2–4.9% (Table III–X).

Lab-to-lab reproducibility

Preliminary experiments revealed that among the many operating parameters involved, the apparent pH of the mobile phase was the most influential parameter on the repeatability of the method when suitable precautions were taken with regards to the instrument setup. Because the actual levels of the impurities present were not quantitatable, impurities were spiked into the samples prior to analysis rather than during the analyis of the spiked sample (Figure 2A). Reproducibility was assessed lab-to-lab (two different analysts using different chromatographic systems on different days) by spiking authentic samples containing 220 mg NapNa and 120 mg PSEH with a stock solution of the impurities' standards. The spiking was done at 0.1% of the actives in the formulation. The concentrations were 1.20 µg/mL for benzoic acid, benzaldehyde, and ephedrone and 2.20 µg/ml for HNPA, HNIN, NINE, AMN, and EMN. The ranges of the recovery results for lab-to-lab reproducibility was 95–107% for 0.1% of the active label strength (Table XI). The data were subjected to statistical analysis using ANOVA. There was not a statistically significant difference between the two labs at the 95% confidence level.

Stability of analytical solutions

The stability of the NapNa and PSEH impurities in the solution were evaluated by analyzing the standard solution and a solution of sample that was spiked with impurities at 0.1% of the actives. The solutions were aged for 72 h at 4°C, and the results demonstrated that the solutions were stable for these settings (see Table XII).

Conclusion

The described method was found to be linear, accurate, reproducible, rugged, and capable of separating impurities associated with NapNa and PSEH. Thus, the method can be used for the routine analysis of stability samples and the quality control of products containing PSEH, NapNa, or both.

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References

1. *Goodman and Gilman's The Pharmaceutical Basis of Therapeutics*, 9th ed., Macmillan Publishing Company, New York, NY, 1996, p 224.
2. *Pharmaceutical Sciences*. Mack Publishing Co., Easton, PA, 1989, p 1059.
3. *The Merck Index*, 11th ed., Merck & Co., Rahway, NJ, 1986, p 6330.
4. D.B. Moir, N. Beaulieu, N.M. Curran, and E.G. Lovering. Liquid chromatographic determination of naproxen and related compounds in raw materials. *J. Assoc. Off. Anal. Chem.* **73:** 902–904 (1990).
5. J.V. Andersen and S.H. Hansen. Simultaneous quantitative determination of naproxen, its metabolite 6-O-desmethylnaproxen and their five conjugates in plasma and urine samples by high-performance liquid chromatography on dynamically modified silica. *J. Chromatogr. Biomed. Appl.* **115:** 325–33 (1992).
6. *US Department of Health and Human Resources, Food and Drug Administration, Guidance to Industry: Q 2B Validation of Analytical Procedure*. Center for Drug Evaluation and Research, Rockville, MD, 1996.

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