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Ion-pairing high-performance liquid chromatographic method for the determination of 5-aminosalicylic acid and related impurities in bulk chemical

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ABSTRACT

An ion-pairing high-performance liquid chromatographic method has been developed for the determination of 5-aminosalicylic acid (5-ASA) bulk chemical in the presence of thirteen potential synthetic process impurities. In addition, the method is suitable for the determination of the in-process intermediate, 5-nitrosalicylic acid. A selective method was achieved on a Hypersil-BDS reversed-phase column using 1-heptanesulfonic acid sodium salt as the ion-pairing reagent in a 0.08 *M* sodium phosphate buffer (pH 2) containing 0.005 *M* 1-heptanesulfonic acid sodium salt and 0.07 *M* sodium chloride–methanol–tetrahydrofuran (85:11:4, v/v/v) isocratic mobile phase. The method was validated using a multi-day, intra-laboratory protocol. The validation addressed linearity, accuracy, precision, sensitivity, and ruggedness of the method. The validated method characterizes the purity of 5-ASA bulk chemical.

INTRODUCTION

Over the last decade, high-performance liquid chromatography (HPLC) has established itself as the analytical method of choice in the pharmaceutical industry. More specifically, HPLC methods have been developed for the analysis of bulk chemicals, dosage forms, and biological samples. However, HPLC of polar, ionizable compounds often exhibit poor resolution, retention, and/or peak shape under reversed-phase conditions. These problems can be solved using ion-pairing reagents to improve the chromatographic separation. The selectivity that is often attainable along with its wide applicability to many pharmaceutical compounds has made ion-pairing chromatography a powerful analytical tool.

5-Aminosalicylic acid (5-ASA) has been shown to be an effective drug for the treatment of inflammatory bowel disease, ulcerative colitis, and Crohn's disease [1–3]. Several methods describe the determination of 5-ASA in biological samples [4–11] and dosage forms [12,13] utilizing HPLC with various methods of detection (*i.e.*, UV, fluorescence, and electrochemical detection). However, no method describes the purity determination of 5-ASA bulk chemical. The proprietary synthesis of 5-ASA can potentially produce thirteen synthetic process impurities in the final product. Purity testing would require the determination of these potential impurities in 5-ASA as well as the characterization of its in-process intermediate, 5-nitrosalicylic acid (5-NSA). To achieve the high degree of selectivity needed for the analysis of a wide polarity range of compounds, an ion-pairing method was developed and validated for the determination of 5-ASA.

EXPERIMENTAL

Materials

5-ASA and 5-NSA were synthesized at Searle Chemical (Augusta, GA, USA). Authentic samples of the impurities were purchased from the following vendors: 4-aminophenol (4-AP), 2,4-diaminophe-

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nol (2,4-DAP), 2,4-dinitrophenol (2,4-DNP), 2-aminophenol (2-AP), 2,5-dihydroxybenzoic acid (2,5-DHBA), phenol, 3,5-dinitrosalicylic acid (3,5-DNSA), 4-nitrophenol (4-NP), and 2-nitrophenol (2-NP), Aldrich (Milwaukee, WI, USA); salicylic acid (SA), Monsanto (St. Louis, MO, USA); 3-aminosalicylic acid (3-ASA), Sigma (St. Louis, MO, USA); 3-nitrosalicylic acid (3-NSA), Custom (Elmwood Park, NJ, USA). Mobile phase and diluting solvent constituents were obtained from the following vendors: HPLC-grade water, J. T. Baker (Phillipsburg, NJ, USA); methanol and tetrahydrofuran, Burdick & Jackson (Muskegon, MI, USA); 85% phosphoric acid, hydrochloric acid, glacial acetic acid, 50% (w/w) sodium hydroxide solution and sodium chloride, Mallinckrodt (St. Louis, MO, USA); 1-heptanesulfonic acid sodium salt, Sigma; sodium phosphate monobasic monohydrate, 98+%, Aldrich.

Apparatus

Development and validation of the method was conducted utilizing the following chromatographic systems: a Beckman Model 421 controller/Model 100A pump (Fullerton, CA, USA) with a Waters Model 710B WISP autosampler (Milford, MA, USA) connected to a Kratos Model 757 UV detector (Foster City, CA, USA) in series with a Waters Model 481 UV detector. The column was a Hypersil-BDS C_{18} (250 mm × 4.6 mm I.D., 5 μm particle size) obtained from Keystone Scientific (Bellefonte, PA, USA) and heated using FIAtron Model TC-50 controller/Model CH-30 column heater (Milwaukee, WI, USA). The exact temperature of the column was measured with a Digi-Sense type K thermocouple obtained from Cole-Palmer (Chicago, IL, USA). Optimization of the method was performed on a Hewlett-Packard Model 1090 liquid chromatograph equipped with a 1040 diode-array detector (Palo Alto, CA, USA). Hewlett-Packard's optimization software package [*i.e.*, interactive computer optimization of HPLC separations (ICOS)] was utilized for the final optimization of the method. Chromatographic measurements (*i.e.*, retention times, peak areas, etc.) and statistical calculations were made with an in-house chromatographic and statistical data management system.

Chromatographic conditions

The aqueous portion of the mobile phase was prepared by accurately weighing 8.6 g (± 0.1 g) of sodium phosphate, 8.2 g (± 0.1 g) of sodium chloride, and 2.2 g (± 0.1 g) of 1-heptanesulfonic acid sodium salt into a suitable 3-l Erlenmeyer flask. HPLC water (2 l) and 5.75 ml of 85% phosphoric acid were added. The buffer solution (pH 2) was mixed well and filtered through a 0.45-μm Nylon filter. Appropriate amounts of methanol and tetrahydrofuran were added for a mobile phase composition of 0.08 *M* sodium phosphate buffer (pH 2) containing 0.005 *M* 1-heptanesulfonic acid sodium salt and 0.07 *M* sodium chloride–methanol–tetrahydrofuran (85:11:4, v/v/v) which was degassed before use. Other pertinent HPLC parameters were as follows: column, Hypersil-BDS C_{18}, 5 μm particle size; flow-rate, 1.5 ml/min; injection volume, 20 μl; column temperature, 35°C; detection, assay, UV at 300 nm and 0.1 a.u.f.s., impurities, UV at 215 nm and 0.1 a.u.f.s.; total run time, 45 min.

Sample preparation

5-ASA samples were prepared by dissolving the desired amount in acidified water (pH 2 using concentrated hydrochloric acid). Dissolution of 5-ASA in the diluting solvent required sonication especially for the more concentrated samples ($\geq$ 0.8 mg/ml).

5-NSA samples were prepared by dissolving the desired amount in a 0.1 *M* acetate buffer at pH 5. Dissolution of the 5-NSA at 1 mg/ml also required sonication.

RESULTS AND DISCUSSION

The HPLC method was developed for the purity testing of 5-ASA bulk chemical. The HPLC method had to ideally resolve thirteen potential synthetic process impurities of different polarity from 5-ASA with the condition that the system be isocratic and able to quantitate 0.1% of each impurity. Due to the wide polarity range of the impurities, an ion-pairing HPLC method was developed. Method development consisted of the selection of an ion-pairing reagent, buffer system, and organic modifiers. Once the selectivity of the method was optimized, performance characteristics were determined through a validation study. Validation addressed

method linearity, accuracy, precision, sensitivity, and ruggedness.

Development/optimization of the HPLC method

The potential synthetic process impurities to be resolved from 5-ASA are shown in Fig. 1. The following ion-pairing reagents were evaluated: 1-hexanesulfonic acid, 1-heptanesulfonic acid, 1-octanesulfonic acid, and 1-dodecylsulfonic acid sodium salt. A plot of the capacity factor as a function of the alkyl chain length of the sulfonic acid sodium salt for 5-ASA, selected potential impurities, and selected nitro compounds are shown in Figs. 2 and 3, respectively. As the alkyl chain of the ion-pairing reagent increased, 5-ASA and its potential impurities retained longer on the column while the nitro compounds retained less. It is conjectured that the ion-pairing reagent causes an ion-exclusion mechanism to occur which decreases the retention of the nitro compounds. 1-Heptanesulfonic acid sodium salt provided a reasonable retention of 5-ASA and its potential impurities; therefore, it was selected as the ion-pairing reagent.

During the initial method development process, a

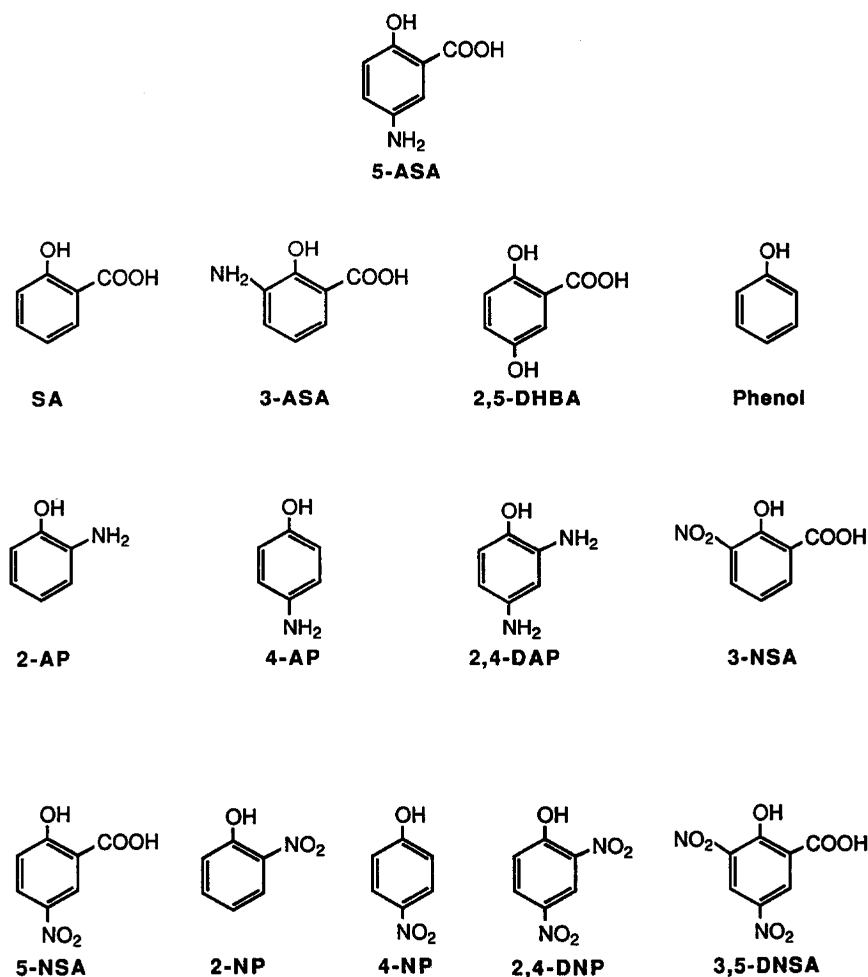

Fig. 1. Structures of 5-aminosalicylic acid (5-ASA) and potential synthetic process impurities: salicylic acid (SA), 3-aminosalicylic acid (3-ASA), 2,5-dihydroxybenzoic acid (2,5-DHBA), phenol, 2-aminophenol (2-AP), 4-aminophenol (4-AP), 2,4-diaminophenol (2,4-DAP), 3-nitrosalicylic acid (3-NSA), 5-nitrosalicylic acid (5-NSA), 2-nitrophenol (2-NP), 4-nitrophenol (4-NP), 2,4-dinitrophenol (2,4-DNP), and 3,5-dinitrosalicylic acid (3,5-DNSA).

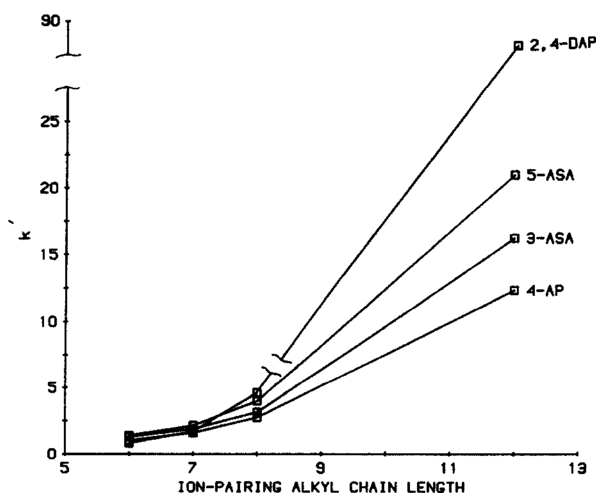

Fig. 2. Capacity factor (k') as a function of the alkyl chain length of the sulfonic acid sodium salt for 5-ASA and selected related impurities.

0.03 *M* sodium acetate buffer system at pH 3 was utilized. Due to the lack of retention of 5-ASA and several impurities at pH 3, the buffer system was adjusted to pH 2 with 85% phosphoric acid. At a sodium phosphate buffer concentration of 0.08 *M* (pH 2), adequate retention of 5-ASA and its potential impurities was obtained; however, the selectivity was not reproducible. It was determined that the phosphate ion plays a role in the selectivity of the method. Therefore, preparation of the buffer system required that the phosphoric acid be added quantitatively to obtain the desired selectivity of the method. Ionic strength was investigated as playing a role in the reproducibility of the method by adding sodium chloride to the buffer system. As a result of these investigations, a 0.08 *M* sodium phosphate buffer (pH 2) containing 0.07 *M* sodium chloride yielded reproducible selectivity.

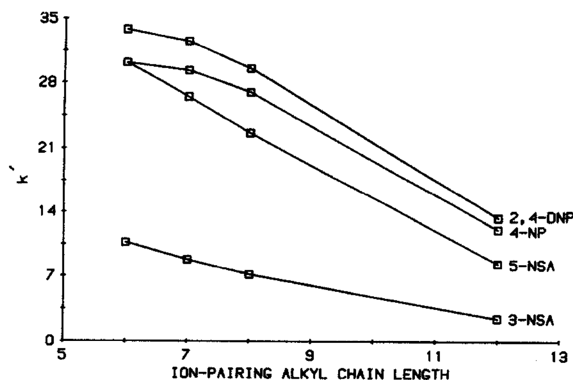

Fig. 3. Capacity factor (k') as a function of the alkyl chain length of the sulfonic acid sodium salt for selected nitro compounds.

Optimization of the organic modifier was performed on a HP 1090 equipped with Hewlett-Packard's optimization software package, ICOS. ICOS utilizes a solvent selectivity triangle and retention mapping as a means of optimizing the separation. Basically, three steps are involved in the program. First, the ratio of buffer to organic modifier for each corner of the triangle is determined so that a similar retention time of 5-ASA is achieved. For this study, methanol, acetonitrile, and tetrahydrofuran were selected as the organic modifiers. Next, the program runs selective percentage combinations of the three corners so selectivity throughout the triangle is fully investigated. Finally, the data are reduced and retention mapping allows the prediction of separations for potential solvent combinations. From the program, several combinations of buffer and organic modifier were selected and experimentally tested. The predicted separations were in excellent agreement with the experimental results. A mixture of 0.08 *M* sodium phosphate buffer (pH 2) containing 0.005 *M* 1-heptanesulfonic acid sodium salt and 0.07 *M* sodium chloride–methanol–tetrahydrofuran (85:11:4, v/v/v) gave the best selectivity of the thirteen potential synthetic impurities in the presence of 5-ASA. The selectivity of the method along with a typical lot of 5-ASA are illustrated in Figs. 4 and 5.

Utilizing the same chromatographic conditions, 5-NSA, an in-process intermediate to 5-ASA, can be profiled. The method's selectivity along with a typical lot of 5-NSA are shown in Fig. 6. The detection of 3-NSA, 3-NP, 2,4-DNP, SA and two unknowns in a lot of 5-NSA chemical demonstrates the method's suitability for the determination of this in-process intermediate. In addition, spectral analyses were performed on 5-ASA and 5-NSA chemical lots utilizing a photodiode-array detector to support further the selectivity of the method.

Validation of the HPLC method

The chromatographic profile of a typical lot of 5-ASA indicated the presence of the impurity 3-

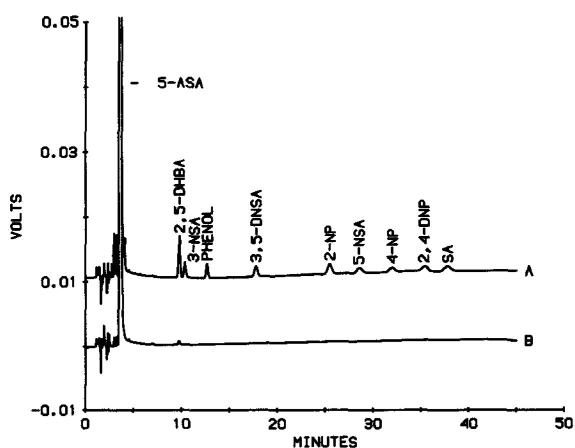

Fig. 4. HPLC of 5-ASA and potential synthetic process impurities at approximately the 0.1% level (A) and a typical lot of 5-ASA (B). Conditions: column, Hypersil-BDS C_{18}, 250 mm × 4.6 mm I.D., 5 μm particle size; mobile phase, 0.08 *M* sodium phosphate buffer (pH 2) containing 0.005 *M* 1-heptanesulfonic acid sodium salt and 0.07 *M* sodium chloride–methanol–tetrahydrofuran (85:11:4, v/v/v); column temperature, 35°C; flow-rate, 1.5 ml/min; injection volume, 20 μl; detection, UV at 215 nm.

ASA at a level of < 0.1%. Therefore, 3-ASA was selected as the impurity for the validation along with 5-ASA. The validation addressed method linearity, accuracy, precision, sensitivity, and ruggedness. The experimental design of the validation consisted of a two run/one analyst/three replicates per

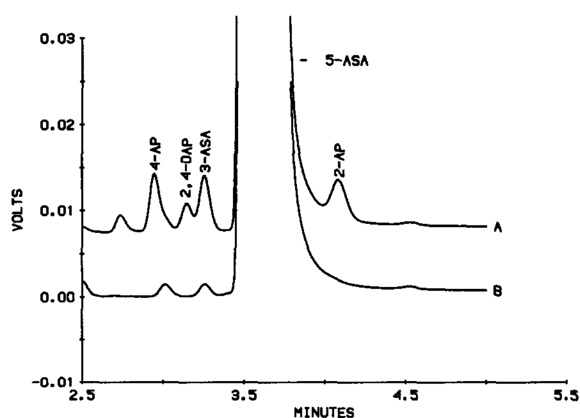

Fig. 5. HPLC from Fig. 4 illustrating the selectivity of the method between 2.5 min and 5.5 min.

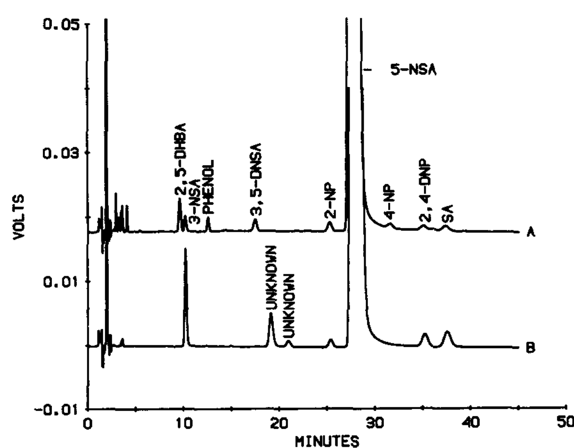

Fig. 6. HPLC of 5-NSA with spiked impurities at approximately the 0.1% level (A) and a typical lot of 5-NSA (B). Conditions as in Fig. 4.

run. For each run of the validation, a system suitability sample was evaluated. The system suitability mixture contains 5-ASA (1 mg/ml), 3-ASA (0.1%), and 2-AP (0.1%). Three parameters from the chromatogram are calculated to ensure that the system is suitable for performing the analyses. At 215 nm, the relative standard deviation (R.S.D.) of the 3-ASA peak-area response recorded for six injections of the system suitability mixture must be less than or equal to 2%. Also, the resolution factors for 3-ASA/5-ASA and for 5-ASA/2-AP must be greater than 2 and 3, respectively. The values of the resolution factors are calculated using the plate count determined from the 3-ASA peak. The linear range investigated for 5-ASA was from 40% to 120% of the target concentration of 1 mg/ml, while at the impurity level, 0.05–1.5% was investigated for 3-ASA. Since linearity of a bulk chemical is being evaluated, three replicates per concentration point were prepared so that accuracy and precision could be addressed at each concentration, concurrently. Data were collected at 215 and 300 nm for 5-ASA, whereas data for 3-ASA were collected only at 215 nm. At 215 nm, the method is sufficiently sensitive to detect 0.1% of each impurity as shown in Figs. 4 and 5. The performance characteristics, based on a single-point standard of the peak areas, for 5-ASA and 3-ASA are given in Tables I and II, respectively. Utilizing a single-point standard at 1 mg/ml for 5-ASA, mean bias recoveries at 215 nm for the

TABLE I

PERFORMANCE CHARACTERISTICS FOR 5-ASA AT 215 nm AND 300 nm UTILIZING A SINGLE-POINT STANDARD AT 1 mg/ml (n = 3)

Concentration level (mg/ml)	Mean bias recovery (%)		Within-run R.S.D. (%)		Between-run R.S.D. (%)		Total R.S.D. (%)	
	215 nm	300 nm	215 nm	300 nm	215 nm	300 nm	215 nm	300 nm
0.4	132.1	100.8	2.2	0.2	0.0	0.4	2.2	0.4
0.6	120.9	100.6	0.5	0.2	1.4	0.3	1.5	0.3
0.8	109.7	100.2	0.6	0.4	0.0	0.0	0.6	0.4
1.0	100.0	100.0	0.3	0.1	0.0	0.0	0.3	0.1
1.2	92.8	99.6	0.5	0.3	0.0	0.4	0.5	0.5

assay concentrations (0.4–1.2 mg/ml) suggest significant error in their determinations. This error, due to the high absorptivity of 5-ASA at 215 nm, causes non-linearity over this concentration range. However, at 300 nm the results show that over the assay concentration range of 0.4–1.2 ml/ml, mean bias recoveries of 99–101% are obtained with excellent precision (total R.S.D.s < 1%). Based on these performance characteristics, 300 nm was selected as the assay wavelength for 5-ASA. Correlation coefficients of 0.9935 and 0.9999 were obtained for 5-ASA at 215 and 300 nm, respectively. The analysis of 3-ASA at 215 nm demonstrated mean bias recoveries of 97–103% and total R.S.D.s less than 1.2% over the range of 0.05–1.5% of the target concentration of 1 mg/ml when utilizing a single-point standard at 0.1%. A correlation coefficient of 0.9997 was obtained for 3-ASA at 215 nm. Therefore, a solution of 5-ASA at 1 mg/ml would be assayed at 300 nm with impurities determined at 215 nm. This would require either a dual-wavelength detector, diode-array detector, or two detectors in series so both wavelengths could be monitored simultaneously and thus allow one injection per sample for both assay and impurity determination.

Finally, method ruggedness was evaluated. This involved investigating columns of different batches and different HPLC instrumentation during the validation. The experimental design of the validation consisted of two runs with a column from a different batch employed for each run. As shown in Tables I and II, the between-run R.S.D.s at the assay (300 nm) and impurity (215 nm) concentrations are less than 0.5% indicating that the method is reproducible when going from column to column. However, when going from one HPLC system to another, two observations were made. First, the temperature setting of the block heaters is not consistent with the actual temperature of the column. A thermocouple connected to the column showed that the

TABLE II

PERFORMANCE CHARACTERISTICS FOR 3-ASA AT 215 nm UTILIZING A SINGLE-POINT STANDARD AT 1 μg/ml (n = 3)

Concentration level (mg/ml)	Mean bias recovery (%)	Within-run R.S.D. (%)	Between-run R.S.D. (%)	Total R.S.D. (%)
0.0005	97.3	0.7	0.0	0.7
0.001	100.0	0.5	0.0	0.5
0.005	102.4	0.8	0.0	0.8
0.01	102.8	0.6	0.0	0.6
0.015	103.1	1.1	0.0	1.1

actual temperature of the column can vary among column heaters. Second, and what is more important, is the temperature of the column needs adjusting to approximately 35°C so the resolution of 5-ASA from 3-ASA and 2-AP is achieved. Although the column temperature can vary from one HPLC system to another, the method is very reproducible once the temperature of the column is adjusted to approximately 35°C and set for that particular HPLC system.

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