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Simple liquid chromatographic method for the analysis of 5-aminosalicylic acid and its degradation product

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Abstract

5-Aminosalicylic acid (5-ASA, mesalamine) is an antiinflamatory agent used in the treatment of Crohn's disease and ulcerative colitis. A simple LC method was developed and validated for the analysis of 5-ASA. The chromatographic separation was achieved on a reversed-phase, C_8 column with UV detection at 290 nm. This isocratic system was operated at ambient temperature and required 8 min of chromatographic time. The mobile phase consisted of methanol–phosphate buffer pH 7.4 (20:80, v/v). The flow-rate was maintained at 1.0 ml/min. Standard curves were linear over the concentration range of 10 to 200 μg ml^{-1}. Intra-day and inter-day relative standard deviations ranged from 1.1–3.5% and 1.2–4.6% respectively. This method was used to: (1) quantify 5-ASA in pharmaceutical formulations without any complex sample extraction procedure, and (2) study the stability of 5-ASA in Sorensen's phosphate buffer (pH 7.4), Sorensen's phosphate buffer containing 5 mg% (w/v) of Carbopol, and in pharmaceutical formulations.

Keywords: 5-aminosalicylic acid

1. Introduction

5-Aminosalicylic acid (5-ASA, Fig. 1) is an anti-inflamatory agent used in the treatment of Crohn's disease and ulcerative colitis. It is available in suppository (Rowasa®, Solvay Pharmaceuticals), capsule (Pentasa®, Marion Merell Dow) and tablet (Asacol®, Procter and Gamble Pharmaceuticals) dosage forms. An LC method with electrochemical detection has been used to investigate the degradation mechanism of 5-ASA in aqueous solution [1]. Liquid chromatographic methods with UV detection have been used to study the stability of this drug in suspension, tablet and suppository dosage forms [2,3]. Stability of 5-ASA and one of its metabolite in plasma has been described by Brendel et al. [4]. Several LC methods have been developed for the analysis of 5-ASA in biological fluids [5–10]. Some of these assay methods used benzene [3] and diethyl ether [5] as the extracting solvents. Separation and quantitation of 5-ASA after derivatization [5] or using ion-pair chromatography [2,6] have also been reported.

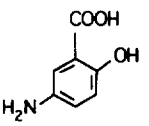

Fig. 1. Structure of 5-aminosalicylic acid.

In our laboratory, we were involved in the de-

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velopment of a bioadhesive 5-ASA suppository formulation used in the treatment of ulcerative colitis. Carbopol-934 was used as a bioadhesive material. The in vitro released studies from these suppositories indicated that incorporation of 2% w/w of Carbopol in the formulation substantially enhanced the release of the drug to the release medium. These in vitro release studies also required the evaluation of the stability of drug in the release medium with and without Carbopol. Therefore, the objectives of this investigation were to: (1) develop a simple, sensitive and specific LC method for the determination of 5-ASA in pharmaceutical formulations without any complex sample extraction procedure, and (2) to study any possible degradation of 5-ASA in aqueous Sorensen's phosphate buffer used as dissolution medium in the in vitro drug release studies.

2. Experimental

2.1. Materials

5-Aminosalicylic acid (Professional Compounding Centers of America, Houston, TX, USA); Rowasa, 500 mg suppository (Solvay Pharmaceuticals, Marrietta, GA, USA); Pentasa, 250 mg capsule (Marion Merell Dow, Kansas City, MO, USA); Asacol, 400 mg tablet (Procter and Gamble Pharmaceuticals, Norwich, NY, USA); 4-acetamidophenol (APAP), *p*-amino phenol (Sigma, St. Louis, MO, USA); water (HPLC grade), methylene chloride, methanol, monobasic potassium phosphate, dibasic sodium phosphate (Fisher, Fairlawn, NJ, USA), Suppocire-AM and Suppocire-AIM (Gattefosse, Westwood, NJ, USA) were used as received.

2.2. Chromatography

The HPLC system consisted of a pump (Model LC-600) programmed by a system controller (Model SCL-6B), an auto injector (Model SIL-9A), a UV–Vis spectrophotometric detector (Model SPD-6AV), and a recorder (Model CR-501), all from Shimadzu (Tokyo, Japan). The separation was carried out on a 25 cm×4.6 mm I.D. S5 C_8 Spherisorb column (Phase Separations, Norwalk, CT, USA). The mobile phase was methanol–phosphate buffer (pH 7.4) (20:80, v/v), with an apparent pH of 7.7. The exact composition of the phosphate buffer used in the preparation of the mobile phase is outlined below. The flow-rate was maintained at 1.0 ml min^{-1}. The column effluent was monitored at 290 nm.

2.3. Solutions

Phosphate buffer (pH 7.4): monobasic potassium phosphate (9.07 g l^{-1}) (solution A) and disodium phosphate (9.48 g l^{-1}) (solution B) were prepared in water (HPLC grade). Solution A (197 ml) was mixed with solution B (803 ml) to make the phosphate buffer.

Mobile phase: methanol (200 ml) was mixed with 800 ml of phosphate buffer. The solution was filtered through a prefilter and a 0.45-μm MAGNA Nylon, 47 mm filter (MSI, Westborough, MA, USA), and degassed in a Branson 2200 sonicator (Branson Ultrasonics, Danbury, CT, USA), for 10 min.

Standard solutions: 5-ASA standard solutions (10 to 200 μg ml^{-1}) were prepared in the mobile phase. The stock standard solution was prepared by dissolving 20 mg of 5-ASA in 100 ml mobile phase in a volumetric flask. Various standard solutions were then prepared from this stock solution after adequate dilution with the mobile phase.

Internal standard solution: APAP solution (160 μg ml^{-1}) was prepared by dissolving 16 mg of APAP in 100 ml of methanol in a volumetric flask.

2.4. Sample preparation for LC

The internal standard solution (60 μl) was added to a borosilicate culture tube and evaporated to dryness at 40°C in an oven. The standard solution or sample to be analyzed (200 μl) was spiked to the test tube and vortexed for 20 s. An aliquot (20 μl) was analyzed by LC.

2.5. Calculation

The ratios of the peak height of 5-ASA to that of the internal standard were calculated. The unknown 5-ASA concentration was determined from the re-

gression equation relating the peak-height ratio (PHR) of the standards to their nominal concentrations.

2.6. Analysis of 5-ASA in suppository formulation

5-Aminosalicylic acid suppositories (Rowasa) contain 22.6% w/w of 5-ASA in a hard fat NF base. A weighed amount of the suppository (220–230 mg) was placed in a 50-ml beaker and dissolved with 1.5 ml of methylene chloride. The dissolved mass was then quantitatively transferred to a 50-ml volumetric flask and the volume was adjusted to 50 ml with mobile phase. This solution was then thoroughly mixed with shaking and sonication for two min. A 5-ml aliquot was then transferred to another 50-ml volumetric flask and the volume adjusted to 50 ml with mobile phase. 5-ASA concentration in the final solution was then determined after filtration through a 0.45-μm MAGNA Nylon syringe filter (MSI, Westborough, MA, USA).

2.7. Analysis of 5-ASA in capsule formulation

The total capsule content (~540 mg) from 5-aminosalicylic acid capsules (Pentasa) were carefully transferred into a 100-ml volumetric flask. A 3-ml volume of methylene chloride was added and the contents were allowed to dissolve. The volume was then adjusted to 100 ml with mobile phase. Serial dilutions were then performed twice to produce a concentration of approximately 100 $\mu g\,ml^{-1}$. The 5-ASA concentration in this final solution was then determined after filtration through a 0.45-μm nylon syringe filter.

2.8. Analysis of 5-ASA in tablet formulation

5-aminosalicylic acid tablets (Asacol) were carefully crushed using a glass mortar and pestle. The crushed tablets were then quantitatively transferred to a 100-ml volumetric flask. The volume was adjusted to 100 ml with the mobile phase and mixed thoroughly with shaking and sonication. A 3-ml aliquot of this solution was then transfered to a fresh volumetric flask and the volume adjusted to 100 ml with mobile phase. The 5-ASA concentration was then determined after filtration through a 0.45-μm nylon syringe filter.

2.9. In vitro release of 5-ASA

In vitro release studies of 5-ASA from the suppositories were carried out in a USP dissolution apparatus 1 [11]. Suppositories were placed in suppository baskets with (900 ml) Sorensen's phosphate buffer (pH 7.4 at 37°C) used as the release medium. At definite time intervals, 1 ml of the release medium was collected and replaced with the same volume of fresh buffer. The 5-ASA content in the release medium was determined by the LC method. The fraction of 5-ASA released was plotted against time to give the release kinetics of the drug from these formulations.

2.10. Saturated solubility studies for 5-ASA

Solubility of 5-ASA in phosphate buffer (pH 7.4) and in phosphate buffer containing 5 mg% of Carbopol was determined at 37°C. Excess drug was added to the solvent and stirred continuously with a magnetic stirrer and kept in a controlled temperature chamber (37°C). After 48 h, the suspension was centrifuged at 2000 rpm for 5 min. The supernatant was collected and concentration of the drug in the supernatant was determined by the LC method.

3. Results and discussion

3.1. Validation of the 5-ASA assay

3.1.1. Linearity

The standard curves were linear over the concentration range of 10.0–200 $\mu g\,ml^{-1}$.The equation of the standard curve relating the peak-height ratio (P) to the 5-ASA concentration (C in $\mu g\,ml^{-1}$) in this range was: $P=0.0308C+0.044$, $r^2>0.999$.

3.1.2. Precision

Within-day precision was determined by analysis of four different standard curves on the same day. Day-to-day precision was determined by the analysis of the same solutions on seven different days over a period of 21 days. During this period, the solutions

were stored under refrigeration (4°C) and solutions for the standard curves were prepared fresh each day from the stock solution. The variability in the peak-height ratio at each concentration was used to determine the precision of the assay procedure and presented in Table 1. Within-day and day-to-day R.S.D. values ranged from 1.1–3.5% and 1.2–4.6%, respectively.

3.1.3. Accuracy

Three quality control samples and the standard solutions were stored under refrigeration at (4°C) over a period of 21 days. These samples were analyzed several times during this period and the accuracy of the assay was determined by comparing the measured concentration to its nominal value (Table 2). The R.S.D. ranged from 0.9–3.2%.

3.1.4. Sensitivity

The sensitivity criteria were determined from seven different standard curves using the lowest limit of reliable assay measurement criteria as described by Oppenheimer et al. [12]. The critical level is the assay response above which an observed response is reliably recognized as detectable. This critical level is also considered a threshold value, defining detection. If the measured value exceeds this value then the presence of an analyte is detected, otherwise it is not. This was 0.73±0.38 μg ml^{-1} (mean±S.D.). The detection level is the actual net response which may a priori be expected to lead to detection. This is the least value of the true concentration that is 'nearly sure' to produce a measured value that results in detection [13]. This was 1.46±0.77 μg ml^{-1} (mean±S.D.). The determination level, the concentration at which the measurement precision will be satisfactory for quantitative determination was 3.86±2.13 μg ml^{-1} (mean±S.D.) for a level of precision of 10% R.S.D.

Table 2
Accuracy in the analysis of 5-ASA in quality control samples

Actual conc. (μg ml^{-1})	Measured conc.[a] (μg ml^{-1})	Accuracy[b]	R.S.D. (%)
20	19.9±0.64	100±3	3.2
79	80.5±0.74	102±1	0.9
140	140±1	100±1	0.9

[a] Mean±S.D.; $n=7$.
[b] Accuracy=(measured concn/actual concn)×100.

3.2. Applications of the LC method

This LC method was used to measure the 5-ASA content in pharmaceutical formulations.

Suppository formulation: Cendrowska et al. [3] have used a LC method for the analysis of 5-ASA in suppository formulation. Their method involved an extraction procedure with benzene prior to LC analysis. Therefore, we decided to develop a method where no extraction procedure will be required for

Table 1
Within-day and day-to-day analytical precision

Within-day[a]			Day-to-day[b]	
Concentration (μg ml^{-1})	Mean peak-height ratio[c]	R.S.D. (%)	Mean peak-height ratio[d]	R.S.D. (%)
0.00	0.00	–	0.00	–
10.0	0.3	3.5	0.3	4.6
40.0	1.2	2.7	1.3	4.2
60.0	1.9	2.3	1.9	3.1
100.0	3.1	1.1	3.1	2.6
120.0	3.6	1.1	3.8	3.2
160.0	5.0	2.2	5.0	2.5
200.0	6.0	2.0	6.1	1.2
Slope	0.031±0.0012	3.7	0.031±0.0004	1.4

[a] Analyzed on the same day.
[b] Analyzed on seven different days within a period of 21 days.
[c] Mean; $n=4$.
[d] Mean; $n=7$.

sample preparation. The accuracy of this method in determining the 5-ASA concentration in the suppository formulation was then determined by comparing the nominal concentration to that of the determined concentration. The determined concentration from three separate batches of suppositories ranged from 101–106% of the nominal value. As reported by Cendrowska et al. [3], our method also didn't notice any appreciable degradation product in the chromatogram. The analysis of 5-ASA in the suppository formulation required a sample filtration step through a 0.45-μm Nylon syringe filter prior to injection into the LC system. The loss of drug, if any, during this process was then evaluated. Standard solutions were injected into LC system prior to and after filtration through 0.45-μm Nylon filters. The absolute peak heights of the standard solutions were compared. The results indicate that the filtration step does not have any influence on the absolute peak height of the drug.

Capsule and tablet formulations: this method was used to determine the concentration of 5-ASA both in capsule and tablet formulations. In the case of the capsules, the capsule contents were dissolved in methylene chloride prior to the addition of the mobile phase. However, the tablets were only crushed in a glass mortar and pestle prior to the addition of the mobile phase. Previously published methods for the analysis of 5-ASA in suppository and tablet formulations didn't produce satisfactory results when used for the capsule formulation. Therefore, development of a new LC method was essential for the analysis of 5-ASA in most commercially available dosage forms. The accuracy of this method in determining 5-ASA content in both capsule and tablet formulations were then determined by comparing the nominal concentration to that of the experimental values. The accuracy values for both capsules and tablets were 99.1±4.7% (mean±S.D.; $n=3$) and 102.2±0.92% (mean±S.D.; $n=3$) respectively.

Stability of 5-ASA in solution: stability of 5-ASA in plasma [4], in dosage forms [2,3] in aqueous solution has been reported [1]. According to Palmsmeier et al. [1], the decomposition of 5-ASA in solution occurs rapidly under conditions promoting oxidation and is most stable under conditions tending to inhibit oxidation. Decarboxylation was reported not to be a major degradation pathway for this drug in aqueous solution. Cendrowska et al. [3] have indicated that the drug is stable in both tablet and suppository formulation. Stability studies of 5-ASA in suspension have also shown that the drug was stable in this dosage form for 90 days at room temperature and under refrigeration [2].

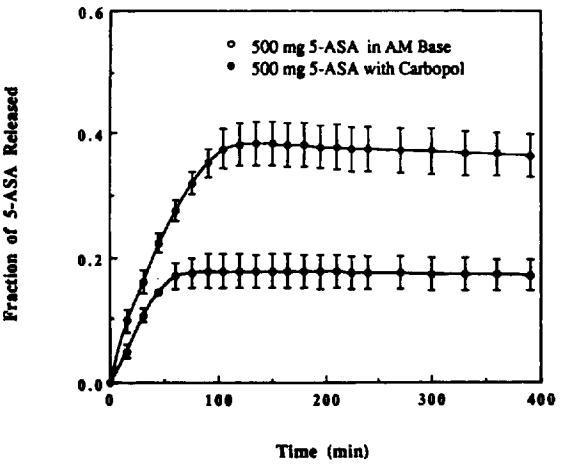

Fig. 2. Fraction of 5-ASA released versus time from suppositories with and without 2% (w/w) of Carbopol in Suppocire–AM base.

The in vitro release of 5-ASA from two different suppository formulations manufactured in our laboratory are shown in Fig. 2 Fig. 3. The results indicated that the fraction of 5-ASA released from suppositories containing 2% w/w Carbopol is substan-

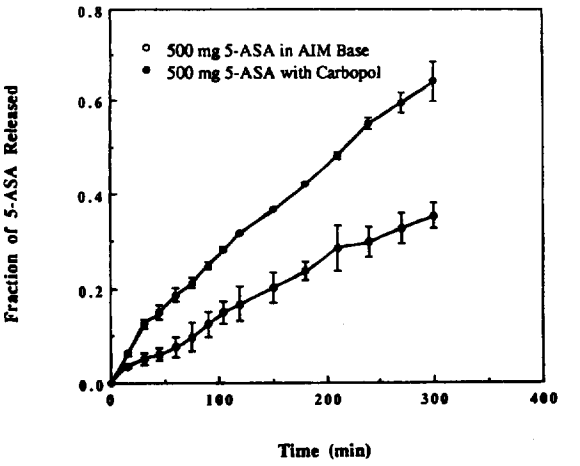

Fig. 3. Fraction of 5-ASA released versus time from suppositories with and without 2% (w/w) of Carbopol in Suppocire–AIM base.

tially higher than that of suppositories without Carbopol. In another experiment, we also evaluated the in vitro release of drug from commercially available 5-ASA suppository formulation in two different dissolution media. The first dissolution medium contained only Sorensens phosphate buffer (pH 7.4) while the second dissolution medium contained 5 mg% of Carbopol in Sorensens phosphate buffer. This 5 mg% of Carbopol in 900 ml of the dissolution medium represented exactly the concentration of Carbopol in the dissolution medium that will be achieved when a suppository containing 2% w/w Carbopol is completely melted. No substantial change in the pH of the medium was noticed after the addition of the 5 mg% of Carbopol. The in vitro release profiles of 5-ASA from this study are shown in Fig. 4. Results from this study further indicated that the fraction of 5-ASA released from Rowosa suppositories into a medium containing Carbopol is higher than a medium containing no Carbopol. This effect of Carbopol on the release was thought to be due to either increase in the solubility of drug, or due to increased stability of the drug in the release medium. The saturated solubility studies indicated that the solubility of 5-ASA in Sorensen's phosphate buffer at 37°C was 10.0±0.2 μg ml^{-1} (mean±S.D.; n=4), where as in phosphate buffer with 5 mg% of Carbopol it was 9.5±0.3 μg ml^{-1} (mean±S.D.; n=4). This study indicated no substantial differences in the solubility of the drug in both the solvents. Therefore, it was quite obvious that in the presence of Carbopol, the stability of the drug in the buffer was affected

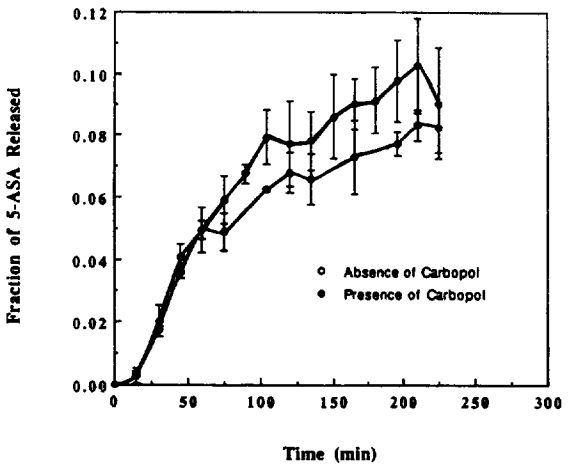

Fig. 4. Fraction of 5-ASA released versus time from Rowasa suppositories into Sorensen's phosphate buffer and buffer containing 5 mg% (w/v) of Carbopol.

A representative chromatogram obtained after the injection of the drug and the internal standard in the mobile phase is shown in Fig. 5a. Fig. 5b and Fig. 5c represent chromatograms obtained after injection of samples from stability studies with 5-ASA in Sorensen's phosphate buffer (pH 7.4) at 37°C. An extra peak, possibly due to the presence of a degradation product was only detected when the drug was present in Sorensen's phosphate buffer for 72 h without any Carbopol (Fig. 5c). However, when the same buffer contained 5 mg% (w/v) of Carbopol-934, no additional peak for the degradation product was detected in the chromatogram within 72 h (Fig. 5b). The

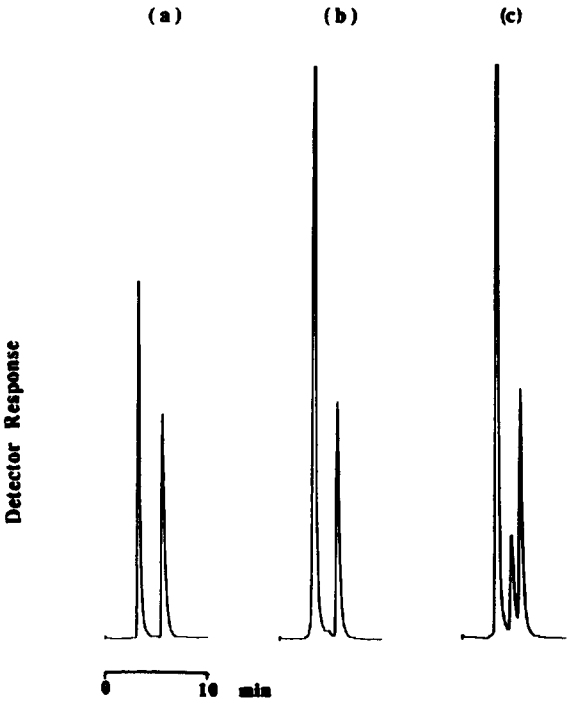

Fig. 5. Representative chromatograms obtained following injection of (a) freshly prepared 5-ASA (60.0 μg ml^{-1}) solution in the mobile phase, (b) 5-ASA (155.2 μg ml^{-1}) solution containing 5 mg% of Carbopol in Sorensen's phosphate buffer (pH 7.4) and kept for 27 h at 37°C, and (c) 5-ASA (150.4 μg ml^{-1}) solution in Sorensen's phosphate buffer (pH 7.4) without Carbopol and kept for 27 h at 37°C. All these solutions contained 19.4 μg ml^{-1} APAP (internal standard).

retention time of this degradation product was found to be identical to that of the para-amino phenol. Presence of para-amino phenol in the sample is a direct indication of the decarboxylation of 5-ASA in Sorensen's phosphate buffer at 37°C. Jivani and Stella [14] have shown that decarboxylation of 4-aminosalisylic acid (4-ASA) in the aqueous solution is the major degradation product for 4-ASA. Palsmeier et al. [1] have shown that degradation of 5-ASA in water occurred mostly by oxidation rather than decarboxylation. However, the result of this study indicated that decarboxylation could be a possible degradation pathway for 5-ASA in Sorensen's phosphate buffer (pH 7.4) at 37°C. Presence of Carbopol (a crosslinked acrylic acid polymer) possibly acts as an inhibitor for this degradation and thereby enhanced the stability of 5-ASA in Sorensen's phosphate buffer.

4. Conclusions

A simple and sensitive method was developed for the analysis of 5-ASA in various commercially available pharmaceutical formulations. The method did not require any complex extraction procedure prior to the LC analysis. This method was able to detect the degradation product for 5-ASA in Sorensen's phosphate buffer (pH 7.4) solution.

Acknowledgments

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