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Short Communication

Determination of 5-aminosalicylic acid and related compounds in raw materials and pharmaceutical dosage forms by high-performance liquid chromatography

C. GANDINI*
Dipartimento di Chimica Farmaceutica, Facoltà di Farmacia, Università di Pavia, Via Taramelli 12, 27100 Pavia (Italy)
G. CACCIALANZA
Istituto di Chimica Farmaceutica e Tossicologica, Facoltà di Farmacia, Università di Pisa, Via Bonanno 6, 56100 Pisa (Italy)
and
M. KITSOS, G. MASSOLINI and E. DE LORENZI
Dipartimento di Chimica Farmaceutica, Facoltà di Farmacia, Università di Pavia, Via Taramelli 12, 27100 Pavia (Italy)
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ABSTRACT

A high-performance liquid chromatographic method using a μBondapak C_{18} column and a UV detector at 230 nm was developed for the simultaneous determination of 5-aminosalicylic acid (5-ASA) and related compounds (4-aminophenol, 3-aminosalicylic acid and 3-aminobenzoic acid). A method for the determination of 5-ASA in pharmaceutical preparations (tablets, granules and rectal suspension) is also described. Calibration graphs for both 5-ASA and related compounds are reported. The standard deviation is ± 0.623 ($n = 10$).

INTRODUCTION

Sulphasalazine has been used extensively in the treatment of ulcerative colitis and Crohn's disease. It consists of sulphapyridine linked to 5-aminosalicylic acid (5-ASA) by an azo bond which is cleaved by gut bacteria to its two main components [1–5].

In recent years, several comparative clinical trials have indicated that 5-ASA is the most likely therapeutic active moiety of sulphasalazine and sulphapyridine is mainly responsible for the side-effects. Therefore, major interest has focused on the

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development of new 5-ASA preparations which are at least as effective as sulphasalazine, but devoid of the adverse reactions caused by sulphapyridine [6–10].

Several methods have been tried to deliver the active 5-ASA to its proposed site of action. They are based on two different principles: (1) prodrugs of 5-ASA in the form of azo compounds have been developed, the cleavage of the azo bond by intestinal bacteria liberating 5-ASA in the intestine [11–14]; and (2) rectal and oral preparations of pure 5-ASA have been designed; special galenical formulations deliver the active compound directly to the intestine [15,16]. The latter approach offers major advantages and for this reason rectal and oral dosage forms of 5-ASA have been developed in recent years.

Numerous high-performance liquid chromatographic (HPLC) methods for the determination of 5-ASA and its major metabolites in body fluids have been reported [17–21], but until now no method has been described for the determination of 5-ASA in pharmaceutical dosage forms and in raw materials in the presence of 5-ASA-related impurities. Related impurities may include 4-aminophenol, 3-aminosalicylic acid and 3-aminobenzoic acid, which are possible degradation products or compounds taken by routes of synthesis (the structures of these compounds are shown in Fig. 1).

This paper reports an HPLC method for the simultaneous separation and determination of 5-ASA and related compounds. A method for the determination of 5-ASA in pharmaceutical dosage forms (tablets, granules and rectal suspension) is also described.

EXPERIMENTAL

Reagents and chemicals

Standards of 5-aminosalicylic acid (5-ASA) and 4-aminophenol (I) were obtained from Merck (Darmstadt, Germany). 3-Aminosalicylic acid (II) and 3-aminobenzoic acid (III) were purchased from Aldrich (Steinheim, Germany), 0.005 *M* PIC B 5 (pentanesulphonic acid) from Waters Assoc. (Milford, MA, U.S.A.) and

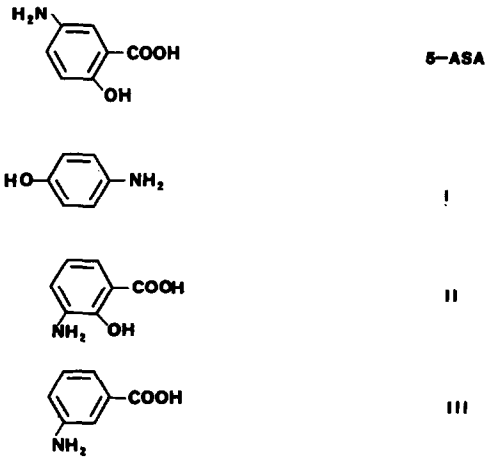

Fig. 1. Structures of 5-aminosalicylic acid (5-ASA), 4-aminophenol (I), 3-aminosalicylic acid (II) and 3-aminobenzoic acid (III).

methanol and acetic acid of HPLC grade from Merck. Raw materials, tablets and granules (Salisofar) were supplied by Sofar (Milan, Italy); rectal suspension (Salofalk) was supplied by Falk (Freiburg, Germany).

Apparatus

The HPLC system used consists of a Varian (Palto Alto, CA, U.S.A.) Model 5060 liquid chromatograph equipped with a Valco semiautomatic sampling valve with 10- and 20-μl loops, a μBondapak C_{18} (10 μm) column (30 × 0.4 cm I.D.), a Varian Varichrom UV–VIS detector and a Varian Vista CDS-401 printer–plotter data system. The mobile phase was 0.005 *M* aqueous PIC B 5 adjusted to pH 3.5 with acetic acid–methanol (90:10, v/v). Isocratic elution was applied at ambient temperature and a flow-rate of 0.7 ml/min. The detector wavelength was 230 nm and the chart speed 0.3 cm/min.

Solutions

The following were prepared: solvent solution A, acetic acid–water (15:85, v/v); standard solution B, 1000 μg/ml 5-ASA in A; standard solution C, 100 μg/ml I, II and III in A; and standard solution D, 500 μg/ml 5-ASA in A.

Limit of detection, linearity and precision

The limit of detection, linearity and precision of the assay were checked using the external standard technique. Standard solutions B and C were diluted to obtain concentrations ranging from 100 to 1000 μg/ml for B and from 20 to 100 μg/ml for C. Triplicate 10-μl aliquots of standard solutions were injected into the chromatograph. Calibration graphs were constructed by plotting ratios of peak area against known concentrations of the compounds. The calibration graphs were linear; the correlation coefficients for 5-ASA and related impurities are reported in Table I. The reproducibility of the chromatographic procedure was established by replicate injections of standard solution D; the mean value was 100.056% with S.D. ± 0.623, and the accuracy expressed as a percentage was 0.056%. For impurities I, II and III the limit of determination was fixed at 20 μg/g of 5-ASA and the limit of detection was 5 μg/g with a signal-to-noise ratio of 2:1.

Procedure for extraction of 5-ASA from pharmaceutical dosage forms

Tablets. Ten Salofalk tablets were crushed to a fine powder in a glass mortar. The powder, equivalent to 400 mg of 5-ASA, was transferred accurately into a 100-ml dark

TABLE I

LINEARITY OF ANALYTICAL PROCEDURE

Compound	Regression equation[a]
5-ASA	$y = 0.369 + 0.400x$; $R = 0.999$
I	$y = 0.278 + 0.976x$; $R = 0.999$
II	$y = 1.38 + 1.595x$; $R = 0.999$
III	$y = -1.52 + 3.41x$; $R = 0.999$

[a] y = Area; x = concentration.

flask with a screw cap and 80 ml of solution C were added. The sample was stirred for 10 min. After decantation, 1 ml of supernatant, previously filtered with a 0.5-μm FHPL PTFE filter (Millipore), was transferred into a 10-ml dark tube and diluted to 10 ml with mobile phase; the nominal concentration of 5-ASA was 500 μg/ml. Triplicate 10-μl aliquots of the test solutions were chromatographed.

Granules. Ten unit-dose packets of granules were crushed to a fine powder in a glass mortar. The powder, equivalent to 1500 mg of 5-ASA, was transferred accurately into a 400-ml dark flask with a screw cap and 300 ml of A were added. The sample was treated as described for tablets.

Rectal suspension. A 15-g amount of rectal suspension, equivalent to 1000 mg of 5-ASA was accurately weighed into a 200-ml volumetric flask. The contents were diluted to volume with solution A and stirred for 10 min. The sample was then treated as described for tablets.

Procedure for extraction of impurities from raw material and pharmaceutical dosage forms

Raw material. The test solutions were prepared by dissolving 1 g of 5-ASA raw material in 10 ml of solution A in a 25-ml dark tube with a screw cap. The contents of the tube were stirred for 10 min and the solution was filtered with a 0.5-μm FHLP PTFE filter. Triplicate 10-μl aliquots of the test solutions were chromatographed.

Tablets. Ten tablets were crushed to a fine powder in a glass mortar. The powder, equivalent to 400 mg of 5-ASA, was transferreed accurately into a 15-ml dark tube with a screw cap and 5 ml of solution A were added. The sample was stirred for 10 min and then treated as for raw material.

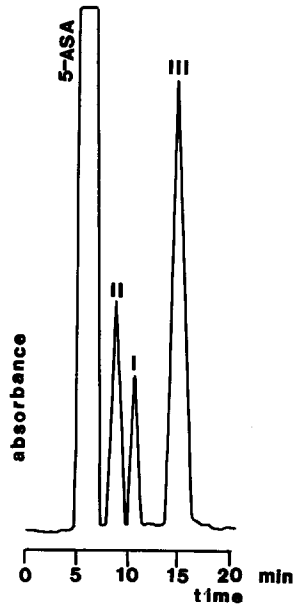

Fig. 2. Chromatogram of a standard mixture of 5-ASA with 0.1% (w/w) of I, II and III (10 μl injected).

TABLE II

DETERMINATION OF 5-ASA IN PHARMACEUTICAL DOSAGE FORMS

Sample	Recovery (% of label claim)		
	Tablets[a]	Granules[a]	Suspension[b]
1	99.85	98.45	98.74
2	100.05	100.36	99.21
3	99.97	99.94	101.36
4	98.70	99.87	98.24
5	101.07	100.96	100.86
Mean ± S.D.	99.93 ± 0.52	99.91 ± 0.61	99.68 ± 1.20
Accuracy (%)	−0.07	−0.09	−0.32

[a] Salisofar.
[b] Salofalk.

Granules. Ten unit-dose packets of granules were crushed to a fine powder in a glass mortar. The powder, equivalent to 1500 mg of 5-ASA, was transferred accurately into a 25-ml dark tube with a screw cap and 15 ml of solution A were added. The sample was then treated as for raw material.

Rectal suspension. A 15-g amount of rectal suspension, equivalent to 1000 mg of 5-ASA, was accurately weighed into a 25-ml dark tube and 5 ml of solution A were added. The sample was treated as for raw material except that triplicate 20-μl aliquots of the test solutions were chromatographed.

RESULTS

Under the described chromatographic conditions, 5-ASA gave a peak with a retention time of 7 min; isocratic elution with the mobile phase used gave a satisfactory resolution of compounds I (10.2 min), II (8.3 min) and III (14.7 min) from each other and from the drug (5-ASA). The resolution of compounds I, II and III from 5-ASA is shown in Fig. 2. Five lots of 5-ASA raw material were examined. For each lot three extractions were made and for each extraction triplicate aliquots were injected. The recovery was 100.106% with S.D. 0.769%.

Three commercial formulations were analysed; the retention times of the 5-ASA in the dosage forms and pure 5-ASA were identical. The procedure for the extration of 5-ASA from commercial dosage forms was excellent, with no unidentified peaks in the chromatogram. The results are presented in Table II.

CONCLUSION

The proposed HPLC method was found to be rapid, sensitive, selective and accurate for the determination of 5-ASA. The method will be useful for routine analysis and for quality assurance of 5-ASA pharmaceuticals dosage forms.

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