Journal of Chromatography, 509 (1990) 195–199
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 203

Note

Investigation of the stability of 5-aminosalicylic acid in tablets and suppositories by high-performance liquid chromatography

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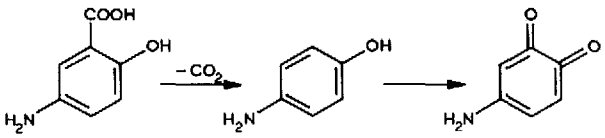
Mesalazine [Fisalamine; 5-aminosalicylic acid (5-ASA)] is a drug used in the treatment of Crohn's disease and ulcerative colitis[1,2] and is produced in two forms coated tablets and suppositories. 5-ASA is very unstable and it is necessary to ensure that a stable final preparation is obtained.

In this paper, an analytical method for the investigation of the stability of tablets and suppositories containing 5-ASA is described and the of influence of light and temperature on the stability of the pharmaceutical forms was studied. Generally, it was assumed that the same decomposition mechanism occurs for 5-ASA as for 4-aminosalicylic acid[3]:



5-ASA (I) II III

Compounds II and III are toxic and their presence in pharmaceutical forms must therefore be carefully controlled.

EXPERIMENTAL

Materials

Mesalazine (Nobel Chemicals, Sweden) was obtained as suppositories containing 250 mg of 5-ASA in 2 g and as tablets containing 250 mg of 5-ASA in 0.4 g.

Apparatus

A Shimadzu (Model UV-160) UV spectrophotometer was used. A modular liquid chromatograph was obtained from LKB, consisting of a Model 250 pump, a Model 2220 integrator, a Model 2151 variable-wavelength detector and a Rheodyne Model 7125 injector. The column (250 cm × 4 mm I.D.) was made of stainless steel and was packed with LiChrosorb RP-18 (10 μm).

Sample preparations for high-performance liquid chromatography (HPLC)

Tablets. A 160-mg amount of powdered tablets (which corresponds to *ca.* 100 mg of active substance) was extracted by shaking with 100 ml of mobile phase for 15 min, then the mixture was centrifuged and the supernatant liquid injected on to the column.

Suppositories. A 2-g amount of powdered suppositories was extracted by shaking with 100 ml of benzene for 15 min and filtered under vacuum. The filter-paper containing the precipitate was dried to constant weight at 40°C, 100 mg of the dry precipitate were dissolved in 100 ml of the mobile phase and portions of the solution were injected onto the column.

UV study of the influence of temperature on the stability of 5-ASA

It was observed visually that suppositories and tablets containing 5-ASA, on prolonged storage at elevated temperatures, changed colour, indicating a decomposition process. The decomposition process may be observed by UV spectrophotometry at 440 nm, because it results in an increase in absorbance at this wavelength (Table I).

Procedure. Dissolve 0.4 g of 5-ASA in a mixture of 10 ml each of ethanol and 0.5 *M* hydrochloric acid and measure the absorbance of the solution at 440 nm in a 1-cm cell.

HPLC investigation of the stability of 5-ASA in its pharmaceutical forms

Spectrophotometry is useful for the qualitative observation of the stability, but it is non-selective and hence unsatisfactory for the determination of the decomposition products. HPLC was therefore used for the investigation of the stability. Two separation systems were used, one to separate 5-ASA and salicylic acid (a by-product of the synthesis) and the second to separate 5-ASA and *p*-aminophenol.

System I. The mobile phase was acetonitrile–water (22:78, v/v) + 0.5% acetic acid at a flow-rate of 1 ml/min. Injections were made of 20 μl of a solution containing

TABLE I

STABILITY OF 5-AMINOSALICYLIC ACID IN SUPPOSITORIES AND TABLETS

Sample	*Time of storage (months)*	*Temperature (°C)*	*Absorbance at 440 nm*	*Appearance*
Tablets	0	Room	0.080	White
	6	Room	0.120	White
	12	Room	0.150	White
	6	37	0.357	Cream
	12	37	0.373	Cream
	6	45	0.367	Cream
	12	45	0.514	Cream
Suppositories	0	Room	0.123	White
	6	Room	0.200	White
	12	Room	0.235	White
	6	30	0.224	White
	12	30	0.283	White

TABLE II

DETERMINATION OF 5-AMINOSALICYLIC ACID IN SUPPOSITORIES AND TABLETS BY HPLC

Sample	*Time of storage (months)*	*Temperature (°C)*	*5-ASA (mg per tablet or suppository)*[a]	*Salicylic acid (%)*	*p-Aminophenol (%)*
Tablets	0	Room	251.2	0.1	–
	6	Room	251.0	0.1	–
	12	Room	250.8	0.1	–
	6	37	250.8	0.1	–
	12	37	250.7	0.1	–
	6	45	250.5	0.1	–
	12	45	249.7	0.1	–
Suppositories	0	Room	250.5	0.1	–
	6	Room	250.5	0.1	–
	12	Room	250.0	0.1	–
	6	30	249.8	0.1	–
	12	30	249.7	0.1	–

[a] Precision of the method: concentration of 5-ASA = 249.7–251.2 mg per tablet, mean = 250.85 and standard deviation = 0.589 (n = 9).

1 mg of active substance in 1 ml and 20 μl of a solution containing 0.004 mg of salicylic acid in 1 ml of the mobile phase. UV detection at 300 nm was used and the range was 0.04 a.u.f.s.

System II. The mobile phase was methanol–0.05 *M* NaH_2PO_4–0.025 *M* Na_2HPO_4–tetrabutylammonium phosphate (12:44:44:1, v/v) at a flow-rate of 0.6 ml/min. Injections were made of 20 μl of a solution containing 1 g of active substance and 20 μl of a solution containing 0.004 mg of *p*-aminophenol in 1 ml of the mobile phase. UV detection at 229 nm was used and the range was 0.04 a.u.f.s.

The external calibration method was used for the quantitative measurements, and the results are given in Table II.

RESULTS AND DISCUSSION

The analysis of suppositories and tablets by HPLC (system I) is shown in Fig. 1, where the peaks of the active substance (1) and salicylic acid (2) can be seen. The amount of salicylic acid is generally stable, because it is an intermediate in the synthesis of 5-ASA, its presence being due to inefficient purification of the final product. The analysis of the same products using system II (in which *p*-aminophenol can be determined) is shown in Fig. 2.

The results indicate that the tablets and suppositories examined contain no decomposition product, for which the retention time is 7.0 min. This is very surprising, because the presence of this compound as a contaminant was assumed according to the reaction shown. A compound with a retention time of 9.6 min was identified as sodium hydrosulphite ($Na_2S_2O_4$). Its amount is stable, because it is a raw material used in the synthesis. In tablets a peak having a retention time of 26 min was also found. It was identified as cellulose acetate phthalate, added as an excipient to tablets.

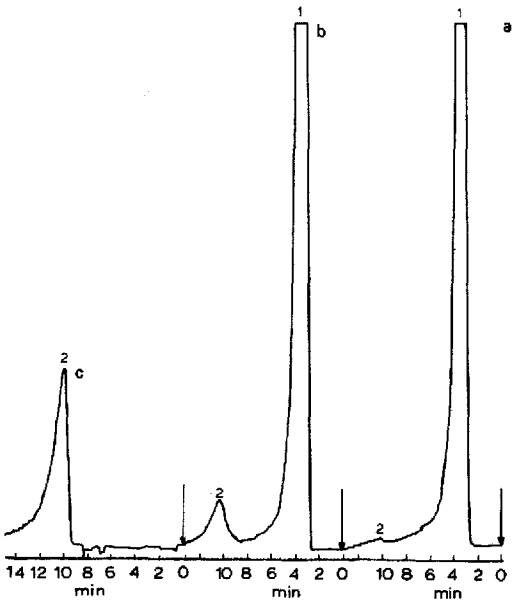


Fig. 1. Chromatograms of 5-ASA in (a) extract of tablets stored for 1 year at room temperature, (b) extract of tablets prepared from inefficient purification of 5-ASA and (c) a standard. Peaks: 1 = 5-ASA; 2 = salicylic acid.

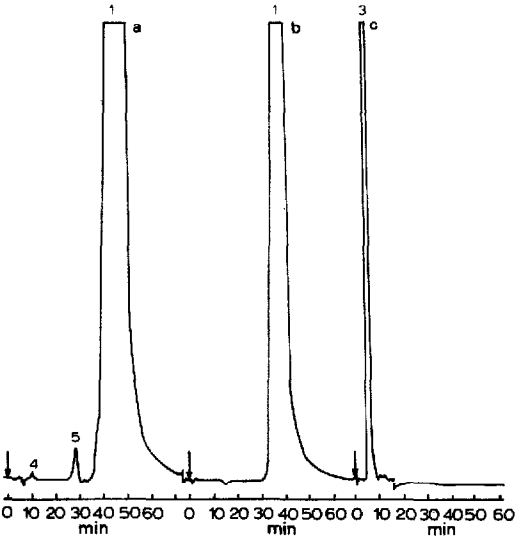


Fig. 2. Chromatograms of 5-ASA in (a) extract of tablets stored for 1 year at room temperature, (b) extract of suppositories stored for 1 year at room temperature and (c) a standard. Peaks: 1 = 5-ASA; 3 = *p*-aminophenol; 4 = sodium hydrosulphite; 5 = cellulose acetate phthalate.

The results indicate that the tablets and suppositories containing 5-ASA are sufficiently stable. The decrease in the content of the active substance in these forms stored at room temperature for 1 year does no exceed 1% and the content of salicylic acid is 0.1%. The HPLC method is sufficiently selective and sensitive for controlling the stability of pharmaceutical forms of 5-ASA.

REFERENCES

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