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SIMULTANEOUS HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ASSAY OF ACETYLSALICYLIC ACID AND SALICYLIC ACID IN FILM-COATED ASPIRIN TABLETS

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SUMMARY

A reversed-phase high-performance liquid chromatography (HPLC) method has been developed for the simultaneous assay of acetylsalicylic acid (I) and salicylic acid (II) in film-coated aspirin tablets. As little as 0.1% II (relative to I) can be quantitatively determined. Using a 5-μm octadecylsilane column with water–acetonitrile–phosphoric acid (76:24:0.5) as the mobile phase enabled the chromatographic separation to be completed in 4 min. Due to the slow rate of decomposition of I to II in the extraction solvent, acetonitrile–methanol–phosphoric acid (92:8:0.5), the analysis of many samples was routinely performed by means of automated HPLC equipment. Other compounds (non-aspirin salicylates, caffeine and acetaminophen) were also separated by the chromatographic system.

INTRODUCTION

The assay of acetylsalicylic acid (I) and salicylic acid (II) in aspirin tablets is a USP XX requirement[1]. If the tablets are coated or buffered, the limit for II is 3%, otherwise the limit is 0.3%. Many liquid chromatographic methods have been reported for the determination of these compounds in formulated products[2–12].

A reversed-phase high-performance liquid chromatography (HPLC) method was developed in this laboratory for the assay of I and II in coated aspirin tablets. The major advantages are: simultaneous assay, speed and resolution of the chromatographic separation, high sensitivity for II, and the ability to analyse many samples with the aid of automated HPLC equipment by minimizing the decomposition of I to II.

EXPERIMENTAL

Materials

Water was purified by means of a Milli-RO®4 and Milli-Q® system (Millipore, Bedford, MA, U.S.A.). Acetonitrile and methanol (both from EM Science, Gibbstown, NJ, U.S.A.) were distilled in glass. Phosphoric acid (85%, Fisher, Fair Lawn,

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NJ, U.S.A.) was reagent grade. Caffeine (Sigma, St. Louis, MO, U.S.A.), acetaminophen (McNeil Consumer Products, Fort Washington, PA, U.S.A.), *o*-salicylsalicylic acid (Pfaltz & Bauer, Stamford, CT, U.S.A.) and acetylsalicylic acid anhydride (Fluka, Happauge, NY, U.S.A.) were used as received. Acetylsalicylic acid and salicylic acid (both from Aldrich, Milwaukee, WI, U.S.A.) were recrystallized.

Instrumentation

A modular liquid chromatographic system consisting of a Model M6000A pump and a WISP automatic sampler, set at 10 μl, were from Waters Assoc., Milford, MA, U.S.A., and a Model LC-85 variable-wavelength detector set at 295 nm from Perkin-Elmer, Norwalk, CT, U.S.A. The detector was interfaced with a 3357 laboratory automation system from Hewlett-Packard, Palo Alto, CA, U.S.A.

Chromatographic conditions

A mobile phase of water–acetonitrile–phosphoric acid (76:24:0.5) was filtered through a 0.45-μm Alpha-450 filter (Gelman Sciences, Ann Arbor, MI, U.S.A.) prior to use. The flow-rate through the 15 cm × 3.9 mm Resolve™ column (Water Assoc.) at 35°C was 2.0 ml/min.

Preparation of standards

An acetylsalicylic acid standard solution of 6.5 mg/ml was prepared in a mixture of acetonitrile–methanol–phosphoric acid (92:8:0.5) (extraction solvent). A salicylic acid standard solution of 0.2 mg/ml (3% relative to I) was prepared in extraction solvent.

Sample preparation

Five tablets were placed in a 250-ml volumetric flask and 150 ml of extraction solvent was added. The sample was sonicated for 15 min and then shaken for 15 min. After dilution to volume, a portion of the solution was transferred to a 50-ml conical centrifuge tube and centrifuged for 15 min at 2000 rpm. A portion of the supernatant was filtered through a 0.45-μm Acrodisc-CR filter (Gelman Sciences).

RESULTS AND DISCUSSION

A representative chromatogram of I spiked with 3% of II is shown in Fig. 1; the separation time was 4 min. Three Resolve™ columns were evaluated and the chromatographic parameters: capacity factor (k'), number of theoretical plates (N), tailing factor (T), relative retention (α), and resolution (R), were experimentally determined and are summarized in Table I. Acetylsalicylic acid anhydride and *o*-salicylsalicylic acid were strongly retained and were eluted after *ca.* 24 min. Caffeine and acetaminophen were eluted near the void volume.

The analytical wavelength of 295 nm was optimized to allow the simultaneous detection of I and II at the same sensitivity. A minimum of 0.1% of II could be quantitatively determined.

The detector response (peak area) was found to be linear over the range 34.8–97.1 μg of I injected and 0.3–3.4 μg of II injected. A linear regression analysis of the data ($n = 10$) resulted in a correlation coefficient of 0.9992 for I and 0.9991 for II.

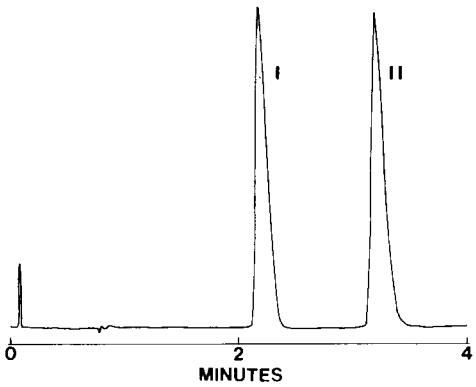

Fig. 1. Representative chromatogram of acetylsalicylic acid (65 μg) spiked with 3% salicylic acid (2 μg).

Six levels of I were added to the placebo to simulate tablets that range in potency from 75 to 127% of label claim. These samples were assayed against an external standard and resulted in an average recovery of 100.2 ± 0.8%. Using the method of standard addition, assays of ten tablet samples spiked with II at levels of 0.8 to 6.2% (relative to I) yielded an average recovery of 105.2 ± 3.3%. No placebo interference was observed.

Fifty replicate injections of a solution containing I spiked with 3% of II performed over a 15-h period resulted in a response with a relative standard deviation (R.S.D.) of 1.5 and 1.3%, respectively. The retention time had an R.S.D. of 0.3% for both analytes.

To determine the overall reproducibility of the method, five replicate assays were performed on each of two typical batches of coated tablets; the results obtained for I were 329.1 ± 4.6 (101.2% of label) and 330.6 ± 1.7 mg/tablet (101.7% of

TABLE I

CHROMATOGRAPHIC PARAMETERS FOR ACETYLSALICYLIC ACID AND SALICYLIC ACID

The definitions of the chromatographic parameters are according to USP XX, page 946.

Compound	*Parameter*	*Column*		
		1	*2*	*3*
Salicylic acid	k'	4.3	4.2	4.5
	N	2478	2444	2752
	T	1.7	1.1	1.3
Acetylsalicylic acid	k'	2.5	2.4	2.6
	N	1250	1376	1953
	T	1.2	1.0	1.1
	α	1.7	1.7	1.7
	R	4.4	4.4	5.2

TABLE II

ASSAY OF COMMERCIAL PRODUCTS

Manufacturer	*Coated*	*mg I per tablet*	*% Label*	*% II**
1	Yes	331.0	101.8	0.12
		333.2	102.5	0.12
2	Yes	323.4	99.5	0.12
		321.1	98.8	0.13
3**	No	397.7	99.4	0.04
		407.7	101.9	0.05
4***	No	331.0	102.2	0.11
		337.1	104.0	0.12
5§	No	255.8	102.3	0.05
		252.7	101.1	0.06

* Relative to acetylsalicylic acid content.
** Contains caffeine.
*** Contains a buffer.
§ Contains acetaminophen and caffeine.

label). Three replicate assays were performed on another sample and the results obtained for II were 0.24 ± 0.01%. To determine the applicability of the method to commercial products, several coated and uncoated tablet formulations were assayed in duplicate. The data are summarized in Table II. Due to the stricter requirement for the content of II in uncoated tablets and the minimal rate of its formation during sample preparation, the method also appears to be suited for this dosage form. The separation of caffeine and acetaminophen in the chromatographic system indicates the potential usefulness for other aspirin formulations.

The extraction solvent, acetonitrile–methanol–phosphoric acid (92:8:0.5), was chosen so as to disintegrate whole tablets in the sample preparation; this minimized any potential sampling problems due to the grinding of the coated tablets. It was found that the tablets did not easily disintegrate if methanol was absent from the extraction solvent.

Earlier work in this laboratory in which 100% methanol was used as the extraction solvent revealed that there was significant decomposition of I to II. From experimental data obtained during an 8-h period, it was determined that salicylic acid was formed at a rate of 0.1% per hour. In extraction solvent, determined over a period of 15 h, the rate was 0.01% per hour. The use of this solvent allowed the method to be automated. All samples could be prepared simultaneously and could be chromatographed by means of the automatic sampler. Preliminary work has indicated that the formation of salicylic acid is even slower in 0.5% phosphoric acid in acetonitrile. Some investigators using reversed-phase HPLC systems have recommended a short time limit between sample preparation and chromatographic analysis[5–7].

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REFERENCES

1 *United States Pharmacopeia,* 20th. rev., U.S. Pharmacopeial Convention, Rockville, MD., 1980, p. 57
2 S. L. Ali, J. Chromatogr., 126 (1976) 651.
3 P. P. Ascione and G. Chrekian, *J. Pharm. Sci.,* 64 (1975) 1029.
4 R. G. Baum and F. F. Cantwell, *J. Pharm. Sci.,* 67 (1978) 1066.
5 V. Das Gupta, *J. Pharm. Sci.,* 69 (1980) 110.
6 V. Das Gupta, *J. Pharm. Sci.,* 69 (1980) 113.
7 R. D. Kirchhoefer and W. E. Juhl, *J. Pharm. Sci.,* 69 (1980) 548.
8 R. D. Kirchhoefer, *J. Pharm. Sci.,* 69 (1980) 1188.
9 V. Y. Taguchi, M. L. Cotton, C. H. Yates and J. F. Millar, *J. Pharm. Sci.,* 70 (1981) 64.
10 C. D. Pfeiffer and J. W. Pankey, *J. Pharm. Sci.,* 71 (1982) 511.
11 P. Helboe, A. K. Kristensen and M. Thomsen, *Arch. Pharm. Chem. Sci. Ed.,* 6 (1978) 141.
12 R. N. Galante, A. J. Visalli and W. M. Grim, *J. Pharm. Sci.,* 73 (1984) 195.