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# QUANTITATIVE DETERMINATION BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF ACETYLSALICYLIC ACID AND RELATED SUBSTANCES IN TABLETS

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## SUMMARY

High-performance liquid chromatography on a Zorbax $C_8$ 7-$\mu$m column (25 cm × 0.46 cm I.D.) with methanol–water–1 *M* phosphoric acid (59:36:5) as the mobile phase has been used for the analysis of several naturally aged batches of fourteen brands of acetylsalicylic acid tablets. The extraction solvent is methanol, containing 2% v/v of formic acid. Salicylic acid is the main impurity. Acetylsalicylsalicylic acid is the second most important impurity, and the corresponding salicylsalicylic acid is rarely present. Buffered or dispersible tablets contain relatively more of the latter two impurities and eventually also the corresponding higher oligomers. Acetylsalicylic anhydride is always a minor impurity. Comparison is made with classical spectrophotometric methods, which are observed to be selective for salicylic acid.

## INTRODUCTION

A number of papers have reported on the analysis of tablets containing actylsalicylic acid (ASA) by high-performance liquid chromatography (HPLC). Early HPLC methods determine salicylic acid (SA) as the sole related substance[1–7]. However, the separation by HPLC of other related substances, such as salicylsalicylic acid (SSA or S2A), acetylsalicylsalicylic acid (ASSA or AS2A) and acetylsalicylic anhydride (ASAN) has frequently been described[8–11], and results for ASA formulations have been reported in several papers[8,12–15]. Other, more recent papers still mention results for SA only[16–18]. HPLC methods for the analysis of analgesic formulations containing several active ingredients are not considered here since their main interest is the determination of the active components and, at most, of SA. Solid-state stability studies and thermal decomposition studies of ASA revealed the formation of higher oligomers, analogous in structure to S2A and AS2A, *i.e.* S3A, S4A and AS3A, AS4A[19,20]. Structures are shown in Fig. 1.

In this paper results are reported for amounts of ASA and related substances in fourteen brands of tablets, available on the Belgian market. Several naturally aged batches of each brand were analysed. Related substances, such as AS3A and S3A,

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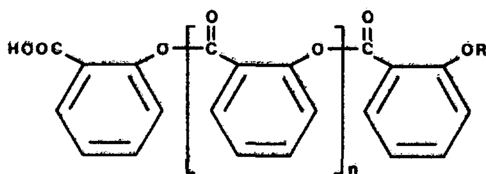



Fig. 1. Structures of some degradation products of acetylsalicylic acid (ASA). R = H: $n$ = 0, S2A; $n$ = 1, S3A; $n$ = 2, S4A. R = $COCH_3$: $n$ = 0, AS2A; $n$ = 1, AS3A; $n$ = 2, AS4A. S2A = salicylsalicylic acid. AS2A = acetylsalicylsalicylic acid.

were found in some samples. A comparison is made with a classical spectrophotometric method. Spectrophotometric methods following the USP[21] and the BP[22] are briefly compared.

## EXPERIMENTAL

### *Samples*

Tablets were obtained from Bayer, Biothera-Asperal, Christiaens, Federa, Nicholas, Reckitt & Colman, Rhône-Poulenc, Riker Benelux, S.M.B., all from Brussels, Belgium. The fourteen brands received the code letters A to N. A scheme showing the composition of the excipients is presented in Table I. Before analysis, tablets were homogenized by milling for two 30-s periods in a small electrical household apparatus.

### *Chemicals and reference products*

Reagents used in spectrophotometric experiments were of pharmacopoeial quality, unless specified otherwise. Chemicals for the preparation of buffers were of *pro analysi* quality (E. Merck, Darmstadt, F.R.G.). Water was glass-distilled. Organic solvents were of at least 99% quality (Janssen, Beerse, Belgium) unless specified otherwise. Methanol used for HPLC was glass-distilled. ASA was purified by crystallization from 2-propanol[23] to obtain a product containing less than 10 ppm SA, as determined by HPLC. Gas chromatography was used to check the absence of 2-propanol. SA was of pharmacopoeial quality. ASAN was obtained by a previously described method[24]: m.p. 82°C, lit. m.p. 85–86°C[24] and 83.5–84.5°C[10]. S2A was obtained from Janssen. AS2A was prepared by acetylation of S2A[10,25] and purified by crystallization from ethanol: m.p. 155°C, lit. m.p. 163.5–164.5°C[10] and 162–164°C[26]. S3A was isolated from a mixture of oligomers obtained in the reaction described for the synthesis of S2A[26] by open column chromatography on silica gel with hexane–ethyl acetate–formic acid (70:30:5) as the mobile phase: m.p. 147°C, lit. m.p. 150–152°C[20]. AS3A was obtained by a previously described method[20]: m.p. 152–153°C, lit. m.p. 161.5–163°C[20]. The structures were further confirmed by mass spectrometry of the methyl esters. Secobarbital, used as the internal standard (I.S.), was purified by repeated crystallization of a commercial sample from diethyl ether–hexane (1:5).

### *Spectrophotometric methods*

A Beckman Model 25 spectrophotometer (Beckman, Fullerton, CA, U.S.A.) equipped with 1-cm cells was used.

*USP-method.* Basically, the method described for aspirin tablets in the USP XX was followed. In the USP XXI this method is still prescribed for aspirin capsules[21]. The method was modified in so far that the limit test was transformed into a quantitative method with a calibration curve for SA, obtained by analysing known amounts of SA: $y = -0.000855 + 0.02300x$, with $y$ = absorbance at 306 nm, $x$ = concentration of SA (μg/ml) in the solution measured, $r = 0.998$.

*BP method.* Basically, the method described for aspirin tablets in the BP 1980, addendum 1983, was used. This method was slightly modified. A powdered tablet sample corresponding to 0.400 g (instead of 0.200 g) of ASA was used (as prescribed in the addendum 1982) to increase the sensitivity. The extraction mixture was sonicated for exactly 3 min. Instead of 1.0 ml of freshly prepared acid ammonium iron-(III) sulphate solution, 2.0 ml were used in order to obtain a linear response over the concentration range 0–25 μg SA/ml. The limit test was thus transformed into a quantitative method with a calibration curve for SA, obtained by analysing known amounts of SA: $y = 0.0037 + 0.00967x$, with $y$ = absorbance at 525 nm, $x$ = concentration of SA (μg/ml) in the solution measured, $r = 0.998$. For buffered or dispersible tablets the extraction was performed with chloroform, as prescribed by the BP for "Dispersible aspirin tablets".

*HPLC method*

*Apparatus and operating conditions.* A Waters Model M-6000 A pump (Waters Assoc., Milford, MA, U.S.A.) was used with a Valco injector Model CV-6-UHPa-N60, equipped with a 20-μl loop (Valco, Houston, TX, U.S.A.), a Pye Unicam Model LC 3 UV variable-wavelength detector, set at 240 nm and 0.08 a.u.f.s. (Pye Unicam, Cambridge, U.K.), a Pye Unicam PD 88 computing integrator and a Kipp Model BD 40 recorder (Kipp & Zonen, Delft, The Netherlands). The column (25 cm × 0.46 cm I.D.) was packed with Zorbax $C_8$ 7 μm (Du Pont, Wilmington, DE, U.S.A.) as described previously[27]. The column was kept at 20°C by means of a water-jacket, as described previously[28]. The mobile phase was methanol–water–1 *M* phosphoric acid (59:36:5), the flow-rate 1 ml/min and the chart speed 5 mm/min. The I.S. solution was a 0.36% m/v solution of secobarbital in methanol.

*Calibration curves.* With the reference substances mentioned above, calibration curves were obtained with $y$ = peak area ratio substance/I.S., $x$ = concentration of substances in the solution injected (mg/ml for ASA, μg/ml for degradation products), $r$ = correlation coefficient, $S_{y,x}$ = standard error of estimate, CR = concentration range examined. ASA: $y = 0.5629 + 13.7351x$, $r = 0.9999$, $S_{y,x} = 0.0074$, CR = 0.3–0.45 mg/ml; SA: $y = 0.0015 + 0.0455x$, $r = 0.9999$, $S_{y,x} = 0.0005$, CR = 0.3–6 μg/ml; ASAN: $y = -0.0004 + 0.0518x$, $r = 0.9998$, $S_{y,x} = 0.0007$, CR = 0.2–2 μg/ml; AS2A: $y = -0.0011 + 0.0338x$, $r = 0.9995$, $S_{y,x} = 0.0018$, CR = 0.4–5 μg/ml; S2A: $y = 0.0008 + 0.0492x$, $r = 0.9999$, $S_{y,x} = 0.0004$, CR = 0.2–2 μg/ml. For higher oligomers the calibration curves for the corresponding dimers (AS2A or S2A) were used.

*Sample preparation.* An aliquot of powdered tablets corresponding to 125 mg of ASA was transferred into a 25-ml volumetric flask, the sample was moistened with 0.5 ml of formic acid, methanol was added to the mark and the mixture was sonicated for exactly 3 min at room temperature. The suspension was filtered through paper, and the first 5 ml were discarded. To 10.0 ml of the filtrate in a 25-ml volumetric flask, 5.0 ml of I.S. were added and the solution was further diluted with water. This

TABLE I

COMPOSITION OF THE EXCIPIENTS OF THE TABLETS AS DECLARED BY THE MANUFACTURERS

| *Excipient* | *Manufacturers (1–9) and tablet brands (A–N)* | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *1A* | *2B* | *2C* | *2D* | *3E* | *3F* | *4G* | *4H* | *5I* | *6J* | *7K* | *7L* | *8M* | *9N* |
| Aluminium dihydroxy-aminoacetate | | | | | | | | | | | | | | * |
| Arabic gum | * | | | | | | | | | | | | | |
| Aroma | | | | * | | * | | | | | | | | |
| Calcium carbonate | | | | | | | * | * | | | | | | |
| Carboxymethylcellulose sodium | | * | | | | | | | | | | | | |
| Cellulose acetate phthalate | * | | | | | | | | * | * | | | | |
| Citric acid | | | | | | * | * | * | | | | | | |
| Cochineal red | | | | * | | | | | | | | | | |
| Diethyl phthalate | * | | | | | | | | | * | | | | |
| Dimethyl polysiloxane | | | | | | | | | | | | | | * |
| Dulcin | | | | | | | | | * | | | | | |
| Ethylcellulose | | * | | | | | | | | | | * | | |
| Gelatin | | | | | | | | | * | * | | | | * |
| Glycerin | | | | | | | | | * | | | | | |
| Lactose | | | | * | | | | | | * | | | | |
| Magnesium carbonate | | | | | | | | | | | | | | * |
| Magnesium stearate | * | | | | | | | | * | | | | | |
| Methylparaben | * | | | | | | | | | | | | | |
| Octylphthalate | | | | | | | | | * | | | | | |
| Polysorbate | | | | | | | | | * | | | | | |
| Saccharin sodium | | | | * | | * | * | * | | | | | | |
| Silicon dioxide | | | | | * | | | | | | * | | | |
| Sodium carbonate | | | | | | * | | | | | | | | |
| Sodium hydrogen carbonate | | | | | | * | | | | | | | | |
| Sodium lauryl sulfate | | | | | * | | * | * | | | | | | |
| Starch | * | * | * | * | * | | * | * | * | * | * | * | * | * |
| Talc | * | | | | | | * | * | | | | | * | |
| Titanium dioxide | * | | | | | | | | | | | | | |

solution was injected immediately to obtain a first chromatogram, used for the determination of the degradation products. To 2.0 ml of the filtrate in a 25-ml volumetric flask, 8.0 ml of methanol and 5.0 ml of I.S. were added and the solution was further diluted with water. This solution was injected immediately after preparation to obtain a second chromatogram, used for the determination of ASA.

## RESULTS AND DISCUSSION

### *Spectrophotometric method*

The spectrophotometric methods of the USP and the BP were slightly adapted as described under Experimental. The selectivity of the methods was checked by analysing dilutions of S2A, AS2A and ASAN. For the USP method an aliquot corresponding to 0.3% of the prescribed amount of ASA was used and for the BP method an aliquot corresponding to 0.15%. In all the experiments no significant absorbance was obtained, which means that the spectrophotometric methods only allow the determination of SA and also that the general heading "non-aspirin salicylates" for the corresponding test of the USP is not justified. While carrying out USP experiments it was observed that the quality of the chloroform used influenced the stability of the solution. This is shown in Fig. 2, where the stabilities of ASA in several brands of chloroform are compared. At 320 nm there is no interference from ASA. It has been reported previously that in non-hydroxylic solvents an equilibrium is established between ASA, the mixed acetylsalicylic anhydride and even SA. At 320 nm only the latter is determined[29]. Since acidified chloroform cannot be used in the USP experiment, chloroform distilled over diphosphorus pentoxide was used in all our experiments. While carrying out BP experiments it was observed that the amount of ethanol present in the solution measured must always be identical, since the colour intensity is inversely proportional to the ethanol concentration. The colour intensity is maximal at pH 2.5–3.5. In the experimental conditions, as described above, the pH is 2.5. The extraction time and temperature are important. Therefore the sample is sonicated for exactly 3 min. An extraction time is not prescribed by the BP, but the temperature of the water for dilution is prescribed to be lower than 10°C. When the water is too cold (1–3°C) part of the ASA and part of the SA are coprecipitated and, consequently, low SA concentrations are recorded. A water temperature of 5–10°C

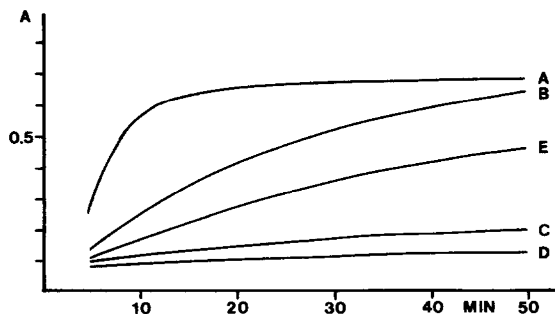



Fig. 2. Absorbance at 320 nm of 0.5% m/v solutions of acetylsalicylic acid in different brands of chloroform. (A) Chloroform containing ethanol; (B) as in A, but distilled; (C) as in B, but acidified with 5% acetic acid, after distillation; (D) as in A, but distilled over diphosphorus pentoxide; (E) chloroform *pro analysi* (E. Merck) containing ethanol.

TABLE II

HPLC METHODS FOR ANALYSIS OF DEGRADATION PRODUCTS IN ACETYLSALICYLIC ACID

Abbreviations: HOAc = acetic acid; $H_3Ci$ = citric acid; EtOAc = ethyl acetate; NM = not mentioned, FLUO = fluorescence.

| *Year (ref.)* | *Sample solvent* | *Stationary phase and column dimensions (mm)* | *Mobile phase* | *Flow-rate* | *Detection (nm)* | *Order of elution of substances separated* | *Time* (min)* |
|---|---|---|---|---|---|---|---|
| 1976 (1) | $CH_3OH$ | Sil-X-I, 37 μm, 500 × 2.6 | Light petroleum**–EtOAc–HOAc (85:15:1) | 1.0 | UV (306) | SA, ASA | 12 |
| 1976 (2) | NM | Sil-X-I, 37 μm, 500 × 2.6 | $CHCl_3$–$n$-$C_7H_{16}$–HOAc (30:65:5) | 0.75 | UV (254) | ASA, SA | 10 |
| 1976 (3) | $CH_3OH$ | LiChrosorb RP-18, 10 μm, 250 × 2.6 | $CH_3OH$(+1% HOAc)–$H_2O$ (48:52) | 1.0 | UV (240) | ASA, SA, AS2A, ASAN | 30 |
| 1976 (8) | $CHCl_3$ + $H_3Ci$ | Spherisorb, 5 μm, 250 × 3 | $CHCl_3$–$n$-$C_6H_{14}$–HOAc (10:87:3) | 2.4 | UV (243) | SA, S2A, ASA, ASAN, AS2A | 8 |
| 1977 (9) | Dioxane–$n$-$C_6H_{14}$ (30:70) | LiChrosorb SI 100, 10 μm, 150 × 4.5 | Dioxane–$C_6H_{14}$ (8:92) saturated with $HClO_4$ (0.1 *M*) | 2.0 | UV (243) | SA, ASAN, ASA, AS2A | 30 |
| 1978 (4) | Dioxane–$n$-$C_6H_{14}$ (10:15) | Amberlite XAD-7, <325 mesh, 150 × 2.8 | $Et_2O$(+0.01 *M* HOAc)–$n$-$C_6H_{14}$ (25:75) | 1.35 | UV (280) | SA, ASA | 10 |
| 1979 (10) | Mobile phase | LiChrosorb RP-18, 10 μm, 250 × 4.6 | $CH_3OH$–$H_2O$–HOAc (53.75:45:1.25) | 1.6 | UV (254) | ASA, SA, AS2A, ASAN, S2A | 17 |
| 1980 (5) | $C_2H_5OH$ | μBondapak $C_{18}$, 10 μm, 300 × 4.0 | $CH_3OH$–phosphate buffer (pH 2.3) (20:80) | 2.0 | UV (300, 254) | ASA, SA | 10 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1980 (6) | Mobile phase | LiChrosorb RP-18, 10 $\mu$m, 250 × 4.6 | $CH_3OH$–$H_2O$(+2.5% HOAc) (54:46) | 1.5 | FLUO | ASA, SA | NM |
| 1980 (12) | Mobile phase | LiChrosorb RP-18, 10 $\mu$m, 250 × 4.6 | $CH_3OH$–$H_2O$–HOAc (51.5:46:2.5) | 1.5 | UV (254) | ASA. SA, AS2A, S2A | NM |
| 1980 (7) | HCOOH–$CH_3OH$ (1:3) | LiChrosorb RP-18, 10 $\mu$m, 250 × 4.6 | $CH_3OH$–$H_2O$–HOAc (52.5:46:1.5) | 2.0 | UV (254) | ASA, SA | 5 |
| 1981 (14) | $CHCl_3$–HOAc (95:5) | Partisil, 10 $\mu$m, 250 × 4.6 | $n$-$C_7H_{16}$–HOAc (95:5) | 3.5 | UV (300) | SA, S2A, ASA, AS2A | 12 |
| 1982 (13) | $CHCl_3$–HOAc (24:1) | Zorbax Sil, 6 $\mu$m, 150 × 4.6 | $n$-$C_6H_{14}$–$CHCl_3$–HOAc (87:10:3) | 2.3 | UV (243) | SA, S2A, ASA, ASAN, AS2A | 20 |
| 1982 (11) | $CH_2Cl_2$ | Zorbax Sil, 6 $\mu$m, 150 × 4.6 | $n$-$C_6H_{14}$–$CHCl_3$–HOAc (80:19:3) | 3.0 | UV (254) | SA, S2A, ASA, ASAN, AS2A | 8 |
| 1982 (16, 17) | NM | $\mu$Bondapak $C_{18}$, 10 $\mu$m, 300 × 3.9 | $CH_3OH$–buffer (pH 3.4) (10:90) | 2.0 | UV (280, 310) | SA, ASA | 25 |
| 1982 (19) | $CH_3OH$ | Spherisorb ODS, 5 $\mu$m, 250 × 4.6 | $CH_3OH$–$H_2O$–$H_3PO_4$ (60:40:0.02) | 1.4 | UV (285) | ASA, SA, AS2A, S2A | 12 |
| 1983 (20) | NM | RP-$C_{18}$ | $CH_3OH$–$H_2O$–HOAc (57:42:1) | 2.0 | UV (254) | ASA, SA, AS2A, S2A, up to S5A and AS6A | 48 |
| 1984 (15) | HCOOH + $CHCl_3$ + $H_3Cl$ | Zorbax Sil, 6 $\mu$m, 250 × 4.6 | $CHCl_3$–$CH_2Cl_2$–$CH_3CN$–HCOOH (70:30:3:0.4) | 2.0 | UV (300) | ASAN, SA, AS2A, ASA | 12 |
| 1984 (18) | $CH_3CN$–$CH_3OH$–$H_3PO_4$ (92:8:0.3) | Resolve, 150 × 3.9 | $CH_3CN$–$H_2O$–$H_3PO_4$ (24:76:0.5) | 2.0 | UV (295) | SA, ASA | 4 |

* Time required to elute the substances mentioned in this table.

** Boiling range: 40–60°C (ref. 1).

seems to be the proper choice. The BP and USP spectrophotometric methods were compared. A bulk ASA sample was chosen to avoid eventual interference from the variability due to extraction from tablet excipients. BP: 32 experiments, mean 0.052% m/m SA, S.D. 0.0025, R.S.D. 4.8%. USP: 29 experiments, mean 0.063% m/m SA, S.D. 0.0073, R.S.D. 11.7%. The reproducibility of the BP method is better. The mean values are comparable but the USP result is significantly higher. In later experiments with tablets the HPLC method was compared with the BP method; the USP method was not used further.

*HPLC method*

Table II gives a review of the published HPLC methods for analysis of ASA in bulk or in tablets containing no other active ingredients. All the mobile phases contain acid or an acid buffer. Straight-phase silica gel and reversed-phase silica gel have both been used successfully. Arguments in favour of the use of silica gel have been reported[11]. ASA and ASAN are mentioned to be unstable in mixed aqueous–organic solvents. This phenomenon can play a role during the extraction and the pre-injection period in the first place and less so during the short period of chromatography, otherwise a normally shaped peak cannot be obtained for ASAN, ASA and SA; also SA cannot be completely separated from ASA. It will be emphasized below that the use of organic solvents is not a guarantee for stability either. Another argument is that on silica gel the small amount of SA is eluted before, and not immediately after ASA, as in reversed-phase chromatography with acidic mobile phases. This elution pattern allows better separation and lower detection limits. However, on reversed phases, the separation and detection limits for SA seem to be satisfactory as well, as will be confirmed below. On polymer material and with an acidic mobile phase, SA is also eluted before ASA[4]. On reversed phases the elution order can be inverted by increasing the pH slightly[16,17]. This phenomenon is also described elsewhere[30]. At the time that this study was carried out the HPLC methods of the USP XXI were not yet available. Here a reversed-phase column and slightly acidified mobile phases, containing alkylsulphonates, are used. SA is eluted before ASA. Other related substances are not detected with this USP method since they are eluted very slowly. For the analyses described in this paper the use of reversed-phase silica gel and an acidic mobile phase was preferred since there was clear evidence for good separation of all the related substances, even of the higher oligomers[20].

A mixture of ASA, SA, AS2A, S2A and ASAN was analysed on seven reversed-phase columns. The mobile phase was methanol–water–1.0 *M* phosphoric acid [$x$:(95 − $x$):5]. The methanol content ($x$) was adapted in order to improve the separation. The elution sequence was essentially the same on all the columns: ASA, SA, AS2A, ASAN and S2A. Results are given in Table III. Except for the Zorbax $C_8$ column the same columns were also used in another study, where more details about the stationary phases were reported[31]. All the columns except one can be used for the separation of the main impurities SA and AS2A. S2A and ASAN, which are not separated on all columns, are only minor impurities of ASA tablets[12]. Poor separation of S2A and ASAN on $\mu$Bondapak has also been observed previously[10]. For further work the Zorbax $C_8$ column was used. The chromatographic conditions were further examined. It was observed that variation of the 1.0 *M* phosphoric acid concentration between 1% and 10% had no significant influence. At 20°C the separation

TABLE III

COMPARISON OF SOME REVERSED-PHASE COLUMNS EXAMINED FOR THE ANALYSIS OF ACETYLSALICYLIC ACID

| *Stationary phases** | *Percentage methanol (x) in mobile phase*** | *Analysis time (min)* | *Remarks* |
|---|---|---|---|
| Partisil ODS | 40 | 16 | ASA and SA not completely separated |
| R-Sil $C_{18}$ LL | 40 | 32 | |
| μBondapak $C_{18}$ | 55 | 15 | ASAN and S2A not completely separated |
| Zorbax $C_8$ | 60 | 15 | |
| Nucleosil $C_8$ | 40 | 31 | Poor peak symmetry |
| Polygosil $C_8$ | 45 | 12 | |
| LiChrosorb $C_8$ | 45 | 35 | ASAN and S2A not separated |

* See ref. 32 for more details on the columns used.

** The mobile phase is methanol–water–0.1 *M* phosphoric acid [$x$:($95-x$):5]; $x$ is adapted in order to optimize the separation.

between S2A and ASAN was much better than at higher temperatures. At 240 nm, the wavelength chosen for detection, the baseline stability was still satisfactory and the specific absorbance for the degradation products was close to the maximum, while that for ASA was relatively low.

*Sample preparation*

The influence of the milling time on the SA content found was examined for one brand of tablets. When the tablet mass was milled for five 1-min periods instead of two 30-s periods, the SA content (HPLC) rose from 0.078% ($n$ = 3, S.D. = 0.006) to 0.085% ($n$ = 5, S.D. = 0.008). This means that slight differences in milling time certainly have no influence on the SA content. The low R.S.D. reported below for the ASA content of the different tablet brands is an indication of the homogeneity of the samples obtained by this milling technique.

The extraction of the powdered tablets is a very important step in the analysis. In the literature many extraction solvents are reported (Table II). As mentioned above, chloroform (Fig. 2) is not the proper choice. The use of dichloromethane is also reported to give unstable solutions[11]. In mixtures of dioxane–hexane the stability is better[4]. The use of acidified chloroform allows good stability and better release from the tablet excipients[8,14,15]. Methanol and ethanol have been used frequently. The stability of ASA is reported to be better in alcohols than in non-hydroxylic solvents[29]. Aqueous mobile phases are not recommended for extraction[6,10,12]. Acidified alcoholic solutions are reported to ensure good stability[18]. In this laboratory it was observed that the presence of small amounts of water in acidified alcoholic solutions plays a role. Therefore formic acid is a better choice than 85% phosphoric acid. The stability of ASA in a number of extraction solvents was briefly checked in this laboratory by following the absorbance at 320 nm. The results are expressed as % m/m SA, formed after 2 h at room temperature. Chloroform or dichloromethane, *ca.* 1%; alcohol-free chloroform or methanol–phosphoric acid 85% (98:2) or acetonitrile–alcohol-free chloroform–formic acid (99:99:2), *ca.* 0.2%; dioxane–hex-

ane (10:15) or acetonitrile–methanol–phosphoric acid 85% (98:2:0.3), *ca.* 0.1%; methanol–formic acid (98:2), *ca.* 0.05%. Therefore the latter solvent was chosen. Previously methanol–formic acid had already been used successfully for the extraction of tablets containing ASA and other active ingredients[30,32].

The influence of extraction time and mode was also examined. Extraction of powdered tablets by sonication for 3 min gives slightly higher ASA values than mechanical stirring for 5 min, while the SA content is virtually the same. Prolonged sonication (5 min in total) gives the same ASA value and a slightly higher SA value (*ca.* 0.01% more), probably owing to hydrolysis. A sonication time of 3 min was therefore chosen. The good reproducibility of the chromatographic method is demonstrated by the low $S_{y,x}$ values obtained for the calibration curves. For the analysis of the tablets the reproducibility is always poorer, owing to the influence of the extraction step, and varies according to the brand of tablet examined. The chromatographic analysis is performed in two steps. The more concentrated solution is used for the determination of the related substances, and a five times more dilute solution for the determination of ASA. This is necessary because the detector response for ASA in the concentrated solution is not sufficiently linear. This allows increased sensitivity, with detection limits of *ca.* 10 ppm for SA, S2A, AS2A. By using only the more dilute

TABLE IV

ANALYSIS OF PLAIN ACETYLSALICYLIC ACID TABLETS BY HPLC

$n$ = Number of experiments; N.D. = not detected; R.S.D. = relative standard deviation.

| *Manufacturer and tablet brand* | *Claimed validity period and tablet age (month)* | | *ASA (% m/m)* | *SA (% m/m)* | *AS2A (% m/m)* | *ASAN (% m/m)* | *S2A (% m/m)* |
|---|---|---|---|---|---|---|---|
| 2C | 60 | 6 | 100.9 | 0.06 | 0.02 | 0.01 | N.D. |
| | | 24 | 101.2 | 0.08 | 0.02 | 0.01 | <0.01 |
| | | 29 | 102.0 | 0.11 | 0.02 | 0.01 | <0.01 |
| | | 33 | 100.7 | 0.09 | 0.02 | 0.01 | N.D. |
| | Highest R.S.D. ($n$) | | 1.3(4) | 16(4) | 11(3) | 3.7(3) | |
| 2D | 60 | 13 | 95.8 | 0.09 | 0.02 | 0.01 | N.D. |
| | | 26 | 96.2 | 0.09 | 0.02 | 0.01 | N.D. |
| | | 29 | 94.4 | 0.11 | 0.02 | 0.01 | N.D. |
| | Highest R.S.D. ($n$) | | 0.9(3) | 8.4(3) | 11(3) | 6.3(3) | |
| 3E | 60 | 13 | 98.3 | 0.06 | <0.01 | N.D. | N.D. |
| | | 25 | 98.9 | 0.05 | <0.01 | N.D. | N.D. |
| | | 39 | 98.9 | 0.04 | <0.01 | N.D. | N.D. |
| | | 49 | 98.5 | 0.04 | <0.01 | N.D. | N.D. |
| | | 59 | 97.2 | 0.12 | <0.01 | N.D. | N.D. |
| | Highest R.S.D. ($n$) | | 1.2(4) | 20(4) | | | |
| 7K | 60 | 31 | 98.8 | 0.06 | <0.01 | N.D. | N.D. |
| | | 39 | 102.6 | 0.06 | <0.01 | N.D. | N.D. |
| | Highest R.S.D. ($n$) | | 0.6(3) | 9.5(3) | | | |
| 8M | 60 | 34 | 99.8 | 0.07 | 0.05 | N.D. | 0.01 |
| | | 52 | 99.4 | 0.05 | 0.04 | N.D. | N.D. |
| | Highest R.S.D. ($n$) | | 0.7(3) | 12(3) | 3.0(3) | | 40(3) |

solution, these limits would be five times higher, which is still sufficient for routine analysis of tablets.

*Analytical results*

The tablets examined can be divided into three groups: plain acetylsalicylic acid tablets, coated tablets and buffered or dispersible tablets. HPLC results are reported in Tables IV–VI. All the samples were analysed at least twice. Values smaller than 10 ppm are reported as not detected (N.D.). Values between 10 ppm and 100 ppm are reported as less than 0.01%. For each brand the R.S.D. value for the batch with the highest R.S.D. for that particular substance is mentioned, together with the number of experiments (*n*) carried out.

For plain acetylsalicylic acid tablets (Table IV) it is clear that SA is the main impurity; AS2A is also present in all the samples. S2A is absent in most of the samples and ASAN is present in only two brands, in small amounts, both from the same manufacturer. The SA content increases with tablet age while the other impurities seem to be formed during the synthesis of ASA and during manufacturing of the tablets. It has been reported that AS2A contents in tablets were often higher than in the corresponding bulk ASA[12]. A typical chromatogram is shown in Fig. 3. Formic

TABLE V

ANALYSIS OF COATED ACETYLSALICYLIC ACID TABLETS BY HPLC

*n* = Number of experiments; N.D. = not detected; R.S.D. = relative standard deviation.

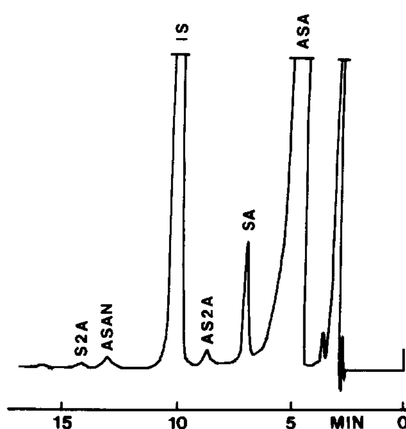
| *Manufacturer and tablet brand* | *Claimed validity period and tablet age (months)* | | *ASA (% m/m)* | *SA (% m/m)* | *AS2A (% m/m)* | *ASAN (% m/m)* | *S2A (% m/m)* |
|---|---|---|---|---|---|---|---|
| 1A | 60 | 9 | 96.5 | 0.12 | 0.03 | N.D. | <0.01 |
| | | 25 | 98.9 | 0.18 | 0.05 | N.D. | <0.01 |
| | | 33 | 98.1 | 0.13 | 0.06 | N.D. | <0.01 |
| | | 40 | 97.3 | 0.17 | 0.21 | N.D. | 0.02 |
| | | 57 | 99.1 | 0.30 | 0.15 | N.D. | 0.02 |
| | Highest R.S.D. (*n*) | | 2.0(4) | 8.2(3) | 1.4(3) | | |
| 2B | 60 | 16 | 101.7 | 0.30 | 0.04 | 0.02 | <0.01 |
| | | 54 | 98.7 | 0.63 | 0.03 | 0.01 | 0.01 |
| | | 59 | 95.8 | 0.79 | 0.03 | 0.01 | 0.02 |
| | Highest R.S.D. (*n*) | | 1.6(4) | 2.3(3) | 20(3) | 9(3) | 11 |
| 5I | 36 | 32 | 101.3 | 0.61 | 0.01 | N.D. | N.D. |
| | | 37 | 100.8 | 0.59 | 0.01 | N.D. | N.D. |
| | Highest R.S.D. (*n*) | | 1.0(3) | 6.4(3) | 20(3) | | |
| 6J | 60 | 30 | 99.7 | 0.32 | 0.02 | 0.01 | N.D. |
| | | 31 | 98.8 | 0.29 | 0.02 | 0.02 | N.D. |
| | | 38 | 99.2 | 0.24 | 0.02 | 0.02 | N.D. |
| | Highest R.S.D. (*n*) | | 0.7(3) | 4.0(3) | 14(3) | 4.8(3) | |
| 7L | 36 | 30 | 98.2 | 0.43 | 0.12 | N.D. | 0.01 |
| | | 33 | 99.7 | 0.43 | <0.01 | N.D. | N.D. |
| | | 36 | 100.3 | 0.47 | <0.01 | N.D. | 0.01 |
| | Highest R.S.D. (*n*) | | 0.9(3) | 2.6(3) | 1.1(3) | | |

TABLE VI

ANALYSIS OF BUFFERED OR DISPERSIBLE ACETYLSALICYLIC ACID TABLETS BY HPLC

$n$ = number of experiments; N.D. = not detected; R.S.D. = relative standard deviation.

| *Manufacturer and tablet brand* | *Claimed validity period and tablet age (months)* | | *ASA (% m/m)* | *SA (% m/m)* | *AS2A (% m/m)* | *ASAN (% m/m)* | *S2A (% m/m)* | *AS3A (% m/m)* | *S3A (% m/m)* | *AS4A (% m/m)* |
|---|---|---|---|---|---|---|---|---|---|---|
| 3F | 36 | 8 | 98.2 | 0.59 | 0.04 | N.D. | N.D. | N.D. | N.D. | N.D. |
| | | 23 | 100.4 | 0.87 | 0.05 | N.D. | <0.01 | 0.01 | N.D. | N.D. |
| | Highest R.S.D. ($n$) | | 1.1(3) | 4.8(3) | 6.0(3) | | | | | |
| 4H | 60 | 13 | 100.4 | 0.11 | 0.05 | N.D. | N.D. | N.D. | N.D. | N.D. |
| | | 26 | 99.3 | 0.41 | 0.10 | N.D. | 0.01 | N.D. | N.D. | N.D. |
| | | 39 | 100.5 | 0.15 | 0.19 | N.D. | 0.01 | <0.01 | N.D. | N.D. |
| | | 51 | 101.7 | 0.19 | 0.24 | N.D. | 0.01 | <0.01 | N.D. | N.D. |
| | Highest R.S.D. ($n$) | | 0.9(3) | 14(3) | 1.8(3) | | 30(3) | | | |
| 4G | 36 | 32 | 100.1 | 0.22 | 0.11 | N.D. | <0.01 | N.D. | N.D. | N.D. |
| | | 40 | 101.8 | 0.17 | 0.24 | N.D. | 0.01 | <0.01 | N.D. | N.D. |
| | | 50 | 101.3 | 0.18 | 0.18 | N.D. | <0.01 | <0.01 | N.D. | N.D. |
| | Highest R.S.D. ($n$) | | 0.4(3) | 3.6(3) | 0.7(3) | | | | | |
| 9N | 60 | 10 | 102.2 | 1.44 | 0.46 | N.D. | 0.05 | 0.32 | 0.03 | 0.16 |
| | | 24 | 99.4 | 2.79 | 0.47 | N.D. | 0.07 | 0.34 | 0.06 | 0.19 |
| | | 44 | 98.1 | 1.46 | 0.36 | N.D. | 0.07 | 0.32 | 0.07 | 0.20 |
| | | 56 | 83.9 | 8.04 | 0.35 | N.D. | 0.12 | 0.16 | 0.07 | 0.10 |
| | Highest R.S.D. ($n$) | | 0.8(3) | 3.7(3) | 2.5(3) | | 6.1(3) | 2.5(3) | 18(3) | 15.5(3) |



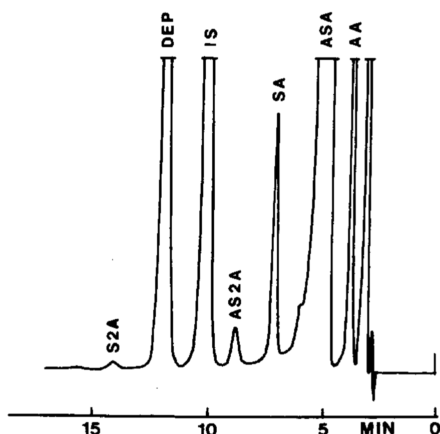




Fig. 3. Typical chromatogram of a plain acetylsalicylic acid tablet (brand 2C, 29 months old; see also Table IV). Peaks: ASA = acetylsalicylic acid; SA = salicylic acid;; AS2A = acetylsalicylsalicylic acid; I.S. = internal standard; ASAN = acetylsalicylic anhydride; S2A = salicylsalicylic acid.

Fig. 4. Typical chromatogram of a coated acetylsalicylic acid tablet (brand 1A, 33 months old; see also Table V). Peaks: AA = acetic anhydride; ASA = acetylsalicylic acid; SA = salicylic acid; AS2A = acetylsalicylsalicylic acid; I.S. = internal standard; DEP = diethyl phthalate; S2A = salicylsalicylic acid.

acid is eluted in the solvent peak. All the batches analysed meet USP XXI or BP 1980 (addendum 1983) requirements for SA (0.3%). The fact that AS2A is a more important impurity than S2A or ASAN corresponds to earlier statements[11–13].

For coated acetylsalicylic acid tablets (Table V) the SA contents are distinctly higher than for plain tablets. The amounts of the other impurities are comparable with those in Table IV. A typical chromatogram is shown in Fig. 4. Tablets A, I and J are completely coated whereas B and L are microencapsulated. All the tablets of the first group contain acetic anhydride (AA), which is eluted between the solvent peak and ASA. The amount of AA increases with tablet age. On the tailing of the ASA peak a small rider peak is observed in several brands of this group. This peak also occurs in a mixture of ASA and AA, and probably corresponds to the mixed acetic acetylsalicylic anhydride. Diethyl phthalate, which is used as a solvent for cellulose acetate phthalate, is sufficiently separated from all ASA derivatives. It was found in brands A, I and J. Its presence in brand I was not declared by the manufacturer (Table I). Other tablet components, such as dulcin (brand I) and methylparaben (brand A), are not separated from ASA and can cause overestimation of the active ingredient. It is not clear why the sweetening agent dulcin is present in a coated tablet formulation. In a more recent formulation dulcin has been omitted by the manufacturer. All the batches examined largely meet USP XXI requirements (3.0%). The BP 1980 (addendum 1983) does not mention a specific monography for coated acetylsalicylic acid tablets, which therefore are supposed to meet the SA requirement for plain tablets (0.3%). This limit is reached by only some brands (A and J).

Buffered or dispersible acetylsalicylic acid tablets (Table VI) contain more impurities. In this group higher oligomers, such as AS3A, S3A, AS4A, are present. A typical chromatogram is shown in Fig. 5. Here also the amount of non-SA impurities seems to be independent of tablet age but merely due to the manufacturing procedure. The time dependence of the SA content is not obvious for brands H and G, but the

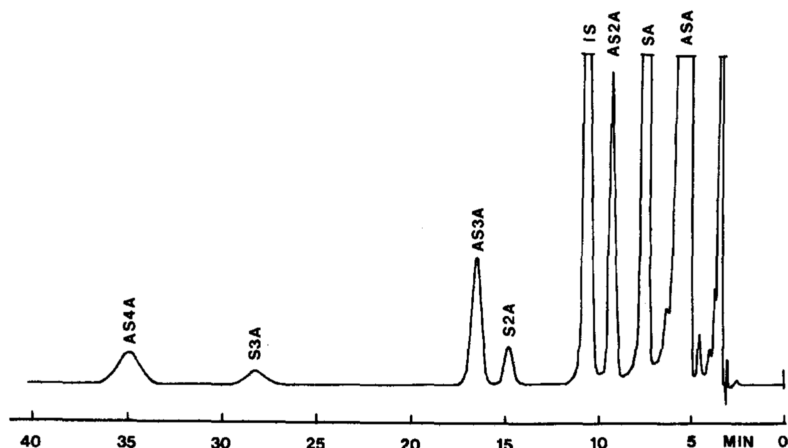



Fig. 5. Typical chromatogram of a dispersible acetylsalicylic acid tablet (brand 9N, 10 months old; see also Table VI). Peaks: ASA = acetylsalicylic acid; SA = salicylic acid; AS2A = acetylsalicylsalicylic acid; I.S. = internal standard; S2A = salicylsalicylic acid; AS3A and AS4A = higher oligomers corresponding to AS2A; S3A = higher oligomer corresponding to S2A.

TABLE VII

COMPARISON OF SALICYLIC ACID CONTENT (% m/m) IN ACETYLSALICYLIC ACID TABLETS AS DETERMINED BY HPLC AND BY A SPECTROPHOTOMETRIC METHOD*

| *Manufacturer and tablet brand*** | *Spectrophotometry* | | | *HPLC* | | | |
|---|---|---|---|---|---|---|---|
| *Plain tablets* | | | | | | | |
| 2C | 0.12 | 0.12 | 0.12 | 0.08 | 0.09 | | |
| 2D | 0.40 | 0.40 | | 0.10 | 0.11 | | |
| 3E | 0.46 | 0.46 | 0.46 | 0.16 | 0.18 | 0.18 | |
| 7K | 0.15 | 0.14 | 0.15 | 0.07 | 0.13 | 0.06 | |
| 8M | 0.35 | 0.35 | 0.35 | 0.31 | 0.31 | | |
| *Coated tablets* | | | | | | | |
| 1A | 0.29 | | | 0.19 | 0.20 | 0.19 | 0.19 |
| 2B | 0.17 | 0.16 | 0.17 | 0.30 | 0.30 | | |
| 5I | 0.41 | 0.40 | 0.41 | 0.59 | 0.58 | 0.65 | |
| 6J | 0.26 | 0.25 | 0.26 | 0.30 | 0.30 | | |
| 7L | 0.22 | 0.25 | 0.23 | 0.46 | 0.48 | | |
| *Buffered or dispersible tablets* | | | | | | | |
| 3F | 0.65 | 0.63 | 0.62 | 0.61 | 0.57 | | |
| 4H | 0.10 | 0.12 | | 0.12 | 0.11 | | |
| 4G | 0.22 | 0.24 | 0.23 | 0.24 | 0.23 | | |
| 9N | 0.21 | 0.24 | | 1.48 | 1.40 | | |

* The modified BP method mentioned in Experimental.

** The batches examined are not always the same as those mentioned in Tables IV and VI.

low SA value can be explained by partial sublimation of SA. All the batches of these brands showed SA whiskers on the tablets. For all the brands of this group the AS2A content is higher than for the other groups (Table IV and V), but never exceeds 0.5%. Brand N even contains considerable amounts of higher oligomers. All the batches except one (9N56) meet the USP XXI SA requirement (3.0%). Two brands only (G and H) meet the BP 1980 (addendum 1983) SA requirement (0.6%). It is not clear why the latter brands, both of the same manufacturer, prepared with the same excipients and apparently of the same stability, have different periods of validity. As already mentioned, the pharmacopoeial methods do not measure the non-SA impurities. The results for brand 9N show that the non-SA impurities can reach *ca.* 1%, even when USP XXI requirements for SA are still met.

For each brand the SA content of one batch was determined by the modified BP method. Results are reported in Table VII. In a number of cases the results are not comparable with the HPLC results. It is clear that some examples of the first two groups that fail the test (0.3%) by the BP method, pass the test by the HPLC method, and *vice versa*. It was demonstrated that cochineal red, present in 2D, interferes with the spectrophotometric method with the result that values are overestimated. No explanation is available for the differences found for 3E. The spectrophotometric method underestimates the SA content in 2B and 5I. Indeed, when the ethanolic extract, prepared for the spectrophotometric analysis, was analysed by the HPLC method, values were obtained close to the HPLC results mentioned in Table V. This suggests that certain tablet excipients interact with the colour reaction. For the buffered or dispersible tablets chloroform has to be used for extraction instead of alcohol. Indeed, when tablets containing citric acid are extracted with ethanol, the ferric ions of the colour reagent are complexed by citric acid, which is soluble in ethanol. The results compare very well except for batch 9N, which does not contain citric acid. The low spectrophotometric value is probably due to strong adsorption of SA on magnesium carbonate or on aluminium dihydroxyaminoacetate[33,34]. This means that the BP method cannot be applied to all types of dispersible acetylsalicylic acid. For aspirin, alumina and magnesia tablets the USP XXI prescribes an extraction solvent containing citric acid and formic acid to assure better extraction of SA.

## CONCLUSION

It can be concluded that both spectrophotometric and HPLC methods can be used to determine SA in ASA tablets. Interaction with tablet excipients can occur in both methods. For the spectrophotometric method the extraction solvent has to be adapted depending on the tablet formulation. HPLC gives a more detailed view of the tablet purity and allows simultaneous identification and quantitative determination of the active ingredient. Therefore, more recent pharmacopoeial methods use HPLC. The method described in this paper is fast and easy to perform and can be used with several brands of reversed-phase columns.

The question can be raised whether different SA limits must be allowed for the different types of ASA tablet. SA is a non-toxic substance, but it can be seen from the results in this study and from elsewhere[6,15,18,35] that buffered tablets with an SA content below 1% can be prepared and stored over a long period. It seems more reasonable then to propose a general limit of 1% SA for all the ASA tablets. The question can also be raised whether the SA content alone gives a sufficient idea of

the ASA tablet impurity. Already a test for AS2A has been introduced in the ASA monography of the Ph. Eur. (0.1% limit)[36]. Since it is known that AS2A can also be formed during tablet manufacturing[8,12], it is logical that the AS2A content in tablets is also limited. The AS2A content seems to be directly related to the content of higher oligomers. From the results of this study and from literature results[12], a limit of 0.5% AS2A seems achievable.

HPLC is the most suitable method for the determination of SA and AS2A in acetylsalicylic acid tablets.

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