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APPLICATION OF GAS–LIQUID CHROMATOGRAPHY AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY TO THE ANALYSIS OF TRACE AMOUNTS OF SALICYLIC ACID, ACETYLSALICYLIC ANHYDRIDE AND ACETYLSALICYLSALICYLIC ACID IN ASPIRIN SAMPLES AND ASPIRIN FORMULATIONS

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SUMMARY

The gas–liquid chromatographic (GLC) determination of salicylic acid (SA) in 12 commercial acetylsalicylic acid (aspirin, ASA) samples and 12 ASA formulations is reported. The GLC determination of SA as an impurity in ASA, utilising methylation with methyl iodide in the presence of potassium carbonate, requires a column chromatographic separation of SA prior to derivatization. Trace amounts of SA in ASA have also been determined by high-performance liquid chromatography (HPLC) on a Sil-X-I adsorption column using light petroleum–ethyl acetate–acetic acid as the mobile phase. Acetylsalicylic anhydride (ASN) and acetylsalicylsalicylic acid (ASSA) were determined by HPLC on a reversed-phase C_{18} column with water–methanol mixtures as the mobile phase. GLC was also applied to the determination of ASN as an impurity in ASA formulations.

INTRODUCTION

The determination of salicylic acid (SA) as an impurity in pure samples of aspirin or acetylsalicylic acid (ASA) is necessary, its presence being limited to 0.05–3% in various pharmacopeiae.

The gas–liquid chromatographic (GLC) determination of SA in ASA utilizes various reagents (HMDC, TMCS, BSTA, BSTFA) for derivitization prior to the actual measurement[1–4]. The use of methylation reagents for esterification such as diazomethane[5] and boron trifluoride[6] has also been reported. The technique used here permits a simple and elegant methylation of both acids, dissolved in a polar solvent such as acetone, with methyl iodide in the presence of potassium carbonate[7]. This method was originally used by Claisen and Eisleb[8] for the preparation of phenyl allyl ethers and has recently been applied for the derivatization of barbituric acids and fatty acids[9].

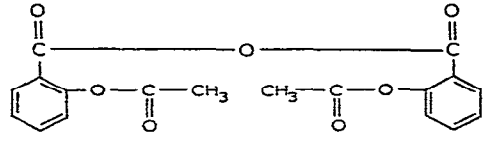
ASA and SA are easily converted quantitatively into their methyl esters in dilute solutions (0.001–0.1 *M*), which could also be confirmed by thin-layer chromatography (TLC) using the solvent system light petroleum (b.p. 40–60°)–ethyl acetate–

acetic acid (85:10:3) and silica gel GF_{254} plates. The method is suitable for the simultaneous determination of ASA and SA by GLC in dilute solutions (0.001 *M*). However, it was found that ASA samples, when analyzed for SA as an impurity, showed a higher SA content than those obtained by spectrophotometry. This result is due to the generation of SA by the slight hydrolysis of ASA during the methylation. The concentration of the ASA solution used here is about 2%, which ultimately leads to its slight hydrolysis on heating at 60–70° in the presence of potassium carbonate. One could not use dilute solutions of ASA, such as 0.001 *M*, for the determination of trace amounts of SA owing to the lack of sensitivity of the method.

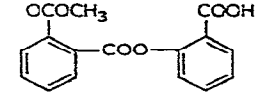
On account of this fact, a column chromatographic separation of trace amounts of SA from ASA is necessary prior to methylation and GLC determination[10]. An iron(III) chloride–urea reagent holds the SA back on a Celite 545 column, where it forms a violet-coloured complex compound with the iron(III) chloride–urea reagent. ASA is first eluted quantitatively with chloroform and later the SA is washed down quantitatively with a mixture of acetic acid and diethyl ether. The resulting solution is then methylated and subsequently gas chromatographed. In this work, this method has been applied to the GLC determination, using camphor as an internal standard, of SA as an impurity in 12 pure ASA samples and 12 ASA formulations containing phenacetin, salicylamide, *p*-acetaminophenol, ascorbic acid, glycocoll, sodium carbonate and salts of calcium and magnesium.

High-performance liquid chromatography (HPLC) is a very useful technique for the separation and detection of SA as an impurity in ASA, as the whole operation is carried out in cold solution without the application of chemical methods for previous separation or derivitization, etc. The sample is not exposed to high temperatures, which would lead to the slight hydrolysis of ASA and produce extra SA that was not previously present in the sample.

There have been reports about the simultaneous determination of aspirin, phenacetin and caffeine by HPLC[11,12]. Henry and Schmitt[13] and Schmitt[14] used an ion-exchange column and separated trace amounts of SA from ASA with an aqueous borate buffer solution of pH 9.2, the ionic strength being adjusted dy the addition of ammonium nitrate. The determination of trace amounts of SA in ASA by HPLC reported here by HPLC utilizes an Sil-X-I adsorption column and light petroleum (b.p. 40–60°)–ethyl acetate–glacial acetic acid (85:14:1) as the mobile phase. The determination was carried on with a Perkin-Elmer Model 1220 liquid chromatograph and an LC-55 UV detector at 306 nm. On a reversed-phase C_{18} column with water–methanol mixtures of different proportions as the mobile phase, SA could not be separated from the bulk of the ASA in order to allow its quantitative evaluation. The optimal conditions for the HPLC determination of SA with the adsorption column were chosen by changing the composition of the mobile phase, flow-rate, column temperature and detection wavelength.



(ASN)



(ASSA)

Acetylsalicylic anhydride (ASN) and acetylsalicylsalicylic acid (ASSA) are considered to be immunogenic impurities in ASA[15,16] and it has been suggested that the allergic reactions of ASA could be caused by ASN and ASSA impurities present in it[15,16]. These allergic reactions are attributed to the presence of antibodies of acetylsalicyloyl or salicyloyl specificity in man following ingestion of ASA. ASN and ASSA are considered to be capable of inducing the formation of acetylsalicyloyl/salicyloyl-specific antibodies in guinea-pigs and rabbits. According to De Weck[15], there is a relationship between the presence of such specific antibodies and the clinically observed allergic reactions.

A spectrophotometric determination of trace amounts of ASN in ASA[17] and a GC determination of ASSA[2] after its derivitization with N,O-bis(trimethylsilyl)-acetamide have been reported. It was reported earlier that the 12 commercial ASA samples analyzed by GLC contained 0.002–0.03 % of ASN and 0.03–0.1 % of ASSA[18]. In this work, ten ASA formulations were analyzed by GLC on a 3 % OV-17 column.

ASN has been also determined in ASA formulations by HPLC after its separation on a reversed-phase C_{18} column with water–methanol (45:55) as the mobile phase and detection at 250 nm. In another experiment, ASN and ASSA were separated from the bulk of the ASA simultaneously by HPLC on a reversed-phase column isocratically with the mobile phase methanol (containing 1 % of acetic acid)–water (48:52) and detection at 240 nm. The ASSA content of four ASA formulations was evaluated by the latter method and the results for ASN were also checked in a few instances with those obtained by the former.

EXPERIMENTAL

Chemicals

All chemicals were of pro analysi grade (Merck, Darmstadt, G.F.R.). Acetone, diethyl ether, chloroform and methanol were dried overnight over anhydrous sodium sulphate. Potassium carbonate was previously dried over phosphorus pentoxide at 170°; methyl iodide was distilled freshly. Iron(III) chloride–urea reagent was prepared freshly by adding 60 g of urea to a mixture of 8 ml of 60 % iron(III) chloride solution and 42 ml of 0.05 *N* hydrochloric acid. Urea was dissolved by swirling and without the aid of heat and the pH of the resulting solution was adjusted to 3.2 by the addition of 6 *N* hydrochloric acid. Celite 545 of particle size 20–45 μm (Serva, Heidelberg, G.F.R.) was used for column chromatography.

Chromatographic column

About 3 g of Celite were intimately mixed in a mortar with 2 ml of iron(III) chloride–urea reagent and the siliceous mass was packed into a 20 × 1.5 cm I.D. chromatographic column, uniform packing being achieved with the aid of a column vibrator. A small piece of glass-wool was inserted previously at the bottom of the column. The chromatographic mass was covered with a small pad of cotton-wool so as to ensure uniform distribution of the solvent over the cross-section of the column.

Phosphate buffer solution, pH 8.0

A 68-g amount of potassium dihydrogen orthophosphate was dissolved in

240 ml of 2 *N* sodium hydroxide solution and the volume was made up with water to 1000 ml.

Phosphate buffer solution, pH 11.3

A 68-g amount of potassium dihydrogen orthophosphate was dissolved in 380 ml of 2 *N* sodium hydroxide solution and the volume was made up with water to 1000 ml.

ASN (m.p. 83°) and ASSA (m.p. 156–158°) were supplied by Bayer (Leverkusen, G.F.R.) and were found to be almost pure by TLC. ASN showed only a very faint spot of ASA. Stock solutions of SA (1 mg/ml in acetone) and ASN (1 mg/ml in benzene) were always freshly prepared. Methanolic solutions of SA, ASN and ASSA (each 1 mg/ml) were prepared for HPLC.

Procedure for GLC of SA in commercial ASA samples and ASA formulations

About 500 mg of ASA or an amount equivalent to 500 mg of ASA in ASA formulation, obtained by powdering and homogenizing 10 tablets, were extracted with cold chloroform for a few minutes, filtered (in the case of formulations) and the volume was made up with chloroform in a 50-ml calibrated flask. A 10-ml volume of this solution was pipetted into the chromatographic column, prepared as described above. The solution was allowed to pass through the column bed; an additional 75–100 ml of chloroform were passed through the column in order to remove ASA completely. This removal was checked by measuring the extinction of the last few millilitres of chloroform at 283 nm. The SA trapped on the column, which could be seen as a violet-coloured band, was then eluted with 15–20 ml of a 1:10 solution of glacial acetic acid in diethyl ether that had previously been saturated with water. The column was washed with an additional 30–50 ml of diethyl ether and the total volume of diethyl ether was extracted three or four times in a separating funnel with 50–75-ml aliquots of water so as to remove acetic acid and any urea present in the organic layer. The organic extract was then dried over anhydrous sodium sulphate, filtered and evaporated to dryness under vacuum at 30–40°. The residue was dissolved in 1 ml of acetone, 1 ml of methyl iodide and 30–50 mg of potassium carbonate were added and the solution was refluxed in a small round-bottomed flask for 20–30 min at 60–70°. The solution was decanted, washed with a small volume of acetone, camphor was added as an internal standard and the volume of the methylated solution was made up to 5 ml with acetone. Aliquots of 1 μl were withdrawn with a Hamilton micro-syringe and injected five or six times directly into the gas chromatograph. The GLC analysis was carried out on a Perkin-Elmer Model F 22 gas chromatograph equipped with a flame-ionization detector and a Hewlett-Packard Model 3380 recorder–integrator–calculator. The instrument contained a 2 m × 4 mm I.D. coiled glass column, packed with 3% OV-17 phenylmethylsilicone, which was coated on Chromosorb G, 80–100 mesh, acid washed and DMCS treated. Nitrogen was used as the carrier gas at a flow-rate of 30–40 ml/min and the column temperature was 110° (isothermal). The standard solutions of the SA were treated in exactly the same manner as the sample, in order to compensate for any losses of SA that occurred during the washing. The amounts of SA present in the pure ASA samples and ASA formulations were obtained from the calculator using the internal standard method. The results for SA in ASA formulations are presented in Table I, and a gas chromatogram is shown in Fig. 1.

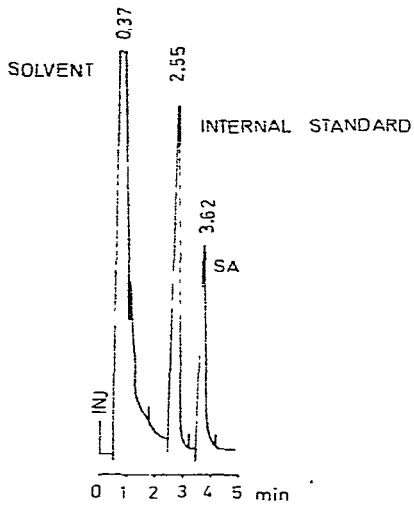


Fig. 1. Gas chromatographic determination of SA in ASA after its column chromatographic separation. Column, 2 m × 4 mm I.D. coiled glass, packed with 3% OV-17 on Chromosorb G AW DMCS, 80–100 mesh; column temperature, 110°, isothermal; injector temperature, 220°; detector temperature, 250°; carrier gas, nitrogen, 35 ml/min.

Procedure for HPLC determination of SA in pure ASA samples and formulations

Ten tablets of the ASA formulation were finely powdered and an amount equivalent to 500 mg of ASA was accurately weighed and extracted for 10 min with 2–3 ml of cold methanol in a stoppered reagent glass, filtered and the volume of the filtrate was made up to 5 ml with methanol. For pure ASA samples, 500 mg were weighed accurately and simply dissolved in 5 ml of methanol. A 5-μl volume of this solution was injected into the liquid chromatograph. After every injection of the sample, the solvent was injected two or three times in order to wash all traces of ASA from the septum and the column. The analysis was carried out on a Perkin-Elmer Model 1220 liquid chromatograph equipped with an LC-55 variable UV detector and a Perkin-Elmer FG1 recorder (1–100 mV). The Sil-X-I adsorption column used had the dimensions 0.5 m × 2.6 mm I.D. and was packed with partly deactivated silica gel particles (37 μm). Other details of the chromatographic conditions are given in Fig. 3. The SA peak area was obtained from the height multiplied by the width at half-height and the SA content of the sample was calculated by the standard addition method.

Procedure for determination of ASN in ASA formulations by GLC

Ten tablets of ASA formulation were powdered and an amount equivalent to 500 mg of ASA was extracted with 40 ml of diethyl ether for 10 min. The ether extract was filtered in a 100-ml round-bottomed flask and the solvent was evaporated under vacuum at 30–40°. The residue was dissolved in 10 ml of phosphate buffer solution (pH 11.3) and the pH of the resulting solution was adjusted to 7.8–8.0. A 0.5-ml volume of benzene solution of ASN standard, containing 400 μg/ml of ASN, was added (the extraction was thereby facilitated). The test-tube was stoppered and shaken vigorously for 10 min and then set aside for few moments so as to permit the separa-

tion of the benzene layer. Each sample was extracted by the addition of further two different amounts of ASN standard solution (150 and 100 μg), but the volume of the benzene added always remained the same (0.5 ml). A 1-μl volume of the supernatant liquid was finally injected into the gas chromatograph. The column used was a 2 m × 4 mm I.D. coiled glass column, packed with 3% OV-17 on Varaport 30, acid washed and DMCS treated. The column was operated at 240° (isothermal).

In another experiment, 10 ml of the cold phosphate buffer solution (pH 8.0) were pipetted into each of three test-tubes, ASN standard benzene solution, containing 200, 150 and 100 μg, respectively, was added (the volume of benzene always being 0.5 ml). They were further treated in the same manner as described above. The amount of anhydride present in ASA formulations was evaluated by the difference of corresponding peak areas of the anhydride standard + 500 mg of sample and the standard solution alone. Reproducible results were obtained by selective extraction with the addition of 200 and 150 μg of ASN standard.

Procedure for HPLC determination of ASN in ASA formulations

An amount equivalent to 500 mg of ASA was extracted with 2–3 ml of methanol in a stoppered reagent glass, filtered and the volume made up to 5 ml with methanol. A 5-μl volume of this solution was injected into Perkin-Elmer Model 1220 liquid chromatograph. A reversed-phase C_{18} column, 0.25 m × 2.6 mm I.D., packed with silica gel SI-100 (10 μm, Merck), coated with chemicaliy bonded C_{18} organic phase, was used for separation. Further details of the chromatographic conditions are presented in Fig. 4. The ASN content of the samples was calculated by the standard addition method. Solutions of the ASN standard were always prepared freshly in methanol.

RESULTS AND DISCUSSION

GLC of SA

Of three columns used (OV-17 on Varaport 30, SE-30 on Varaport 30 and OV-225 on Chromosorb W), the OV-17 column gave the optimal separation. The detection limit for SA lies between 10 and 20 ng. The hydroxyl functional group of the salicylic acid is weakly acidic ($K = 4 \cdot 10^{-14}$) and will not be changed under the experimental conditions to an OCH_3 group. The methylated solutions could be stored for several days provided that moisture is completely excluded.

The column chromatographic isolation of SA from bulk ASA is necessary prior to methylation. The SA thus isolated by the method of Weber and Levine[19,20] is generally determined spectrophotometrically. However, other phenolic compounds, such as salicylamide, *p*-aminophenol and acetaminophen, which also form coloured compounds with the iron(III) chloride–urea reagent, would also then contribute to the absorbance values when eluted from the column along with SA. The methylation of the SA and its subsequent GLC determination has the advantage over spectrophotometry that it is a specific method. Any other species present would not be methylated and, even if derivatized, would have different retention values.

The method applied here eliminates almost completely the possibility of hydrolysis of ASA during the methylation, and measures only the amount of SA present in the sample, not that generated during the course of the chemical operations.

Twelve pure ASA samples, obtained from different manufacturers, contained 0.02–0.05% of SA, which complies with the specifications of most of the pharmacopoeias. Twelve ASA formulations analyzed contained amounts of SA between 0.05 and 6.9% (Table I). The standard deviation of the method was found to be ±0.008 and the coefficient of variation was ±2.59%.

TABLE I

DETERMINATION OF SA CONTENT OF ASA FORMULATIONS

Product	*SA content (%)*	
	GLC	*HPLC*
A as calcium salt	0.13	0.10
B + salicylamide + phenacetin	6.90	6.15
C + salicylamide	6.24	5.87
D + magnesium oxide	3.11	3.23
E	0.06	0.07
F + vitamin C + sodium carbonate	1.67	1.38
G + *p*-acetaminophenol	0.23	0.15
H	0.15	0.13
I	0.25	0.30
K	0.05	0.05
L + glycocoll	0.04	0.05
M	0.43	0.44

HPLC of SA

There was good agreement between the results obtained by GLC and HPLC. With HPLC, it is a great advantage that the sample is simply dissolved in a cold solvent and injected directly. Under the experimental conditions used, the determination of trace amounts of SA in bulk ASA was not affected by the presence of salicylamide, phenacetin, ascorbic acid, *p*-acetaminophenol and salts of calcium and magnesium. The detection wavelength was chosen as 306 nm, where the SA has an absorption maximum. ASA, although present in bulk, would cause negligible absorption (Fig. 2). It is desirable for the minor component to be eluted before the major component in trace analysis. SA eluted from the column before ASA gave a higher accuracy and a greater ease of determination (Fig. 3a).

The important factor of the influence of the mobile phase on the selectivity of the system is evident here. As other columns packed with particles of smaller diameter or with different dimensions were not available, then instead of improving the column efficiency by increasing the number of theoretical plates, the selectivity factor has to be utilized maximally. Fig. 3a shows the separation of SA from bulk ASA after a small amount of acetic acid (1%) had been added to the mobile phase. A good separation was also obtained with formic acid as the strong solvent component of the mobile phase (Fig. 3b), but it was avoided owing to its unpleasent odour.

Without the addition of acetic or formic acid to the mobile phase (light petroleum + ethyl acetate), SA was not eluted or the separation was inadequate if methanol or propanol-2 was added to the solvent mixtures as the polar component. A strong solvent selectivity arises from the solute–solvent–adsorbent interactions, where acetic or formic acid acts as the strong solvent component. The effect of the

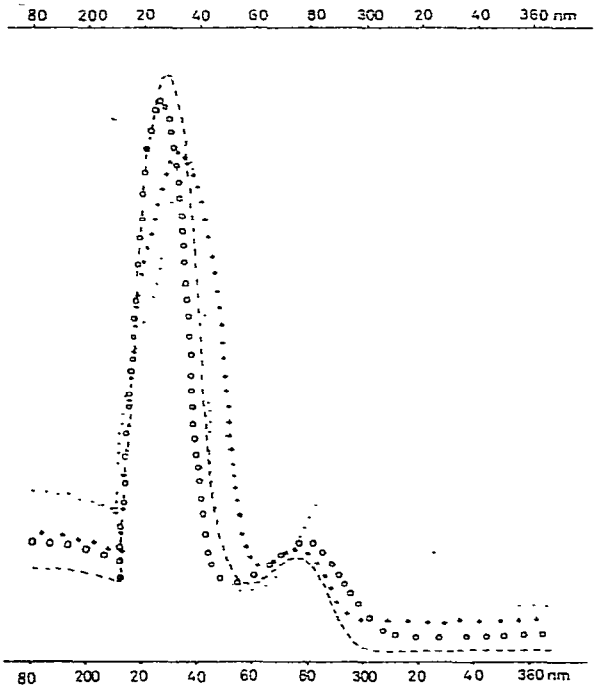


Fig. 2. UV spectra of SA (···), ASA (+++), ASN (○○○) and ASSA (——) taken with a Perkin-Elmer Model 124 recording double-beam UV–visible spectrophotometer. Ethanol was used as solvent to make 0.0004 *M* solutions of SA and ASA and 0.0002 *M* solutions of ASN and ASSA.

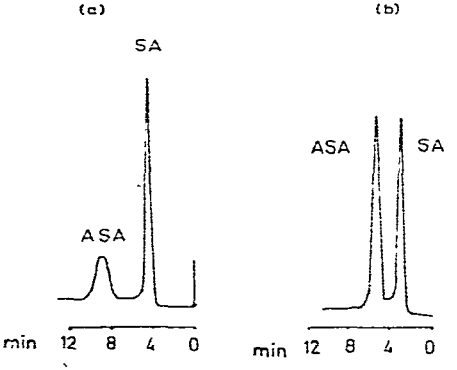


Fig. 3. HPLC separation of trace amounts of SA from bulk ASA. (a) Column, Sil-X-I, 0.5 m × 2.6 mm I.D., partially deactivated; pressure, 400 p.s.i.; flow-rate, 1 ml/min; wavelength, 306 nm; mobile phase, light petroleum (b.p. 40–60°)–ethyl acetate–glacial acetic acid (85:14:1); temperature, ambient; amount injected, 5 μl; recorder sensitivity, 5 mV; chart speed, 12 in./h. (b) Mobile phase, light petroleum (b.p. 40–60°)–ethyl acetate–formic acid (85:10:5); all other parameters as in Fig. 3a.

flow-rate on the elution and separation was also studied. With a flow-rate of 0.5 ml/min, broader peaks were obtained owing to diffusion effects and there was no significant increase in resolution. In order to achieve a rapid analysis with optimal separation, a flow-rate of 1 ml/min and ambient temperature were chosen.

Attempts to shorten the analysis time further by operating at 35 and 40° did not bring the desired results. Also, at higher temperatures hydrolysis of ASA is accelerated owing to its thermal instability. If the same sample had been allowed to stand for 12 h, an increase in SA peak height as a result of gradual hydrolysis was observed. This result was also confirmed by Henry and Schmitt[13] and Schmitt[14]. During the analysis of some of the ASA formulations, a rather rapid increase in SA peak height was noticed. This effect could be attributed to some unknown impurity (auxiliaries present in ASA formulations were not known) extracted along with SA + ASA and thus interacting with the adsorbent and leading to the adsorbent-catalyzed reaction of the sample during the separation process. This produces a minute amount of SA not previously present in the sample. Pure ASA samples analyzed for SA did not show this effect. However, in HPLC the sample is protected from air and light, and by utilizing shorter separation times the reaction time is greatly reduced[21]. It is recommended that the analysis should be performed as quickly as possible after the sample has been brought into solution.

Attempts to separate trace amounts of SA from bulk ASA and to determine it on a reversed-phase column were not successful. The separation of both components with water–methanol (55:45) as the mobile phase was inadequate.

Twelve pure ASA samples analyzed contained between 0.02 and 0.06% of SA; twelve ASA formulations analyzed contained between 0.03 and 6.9% of SA (Table I). The standard deviation was found to be ± 0.065 and the coefficient of variation was $\pm 3.62\%$. The detection limit for SA is about 100 ng, and could be further increased by injecting a larger sample and utilizing a more sensitive recorder setting.

GLC of ASN

The addition of a known amount of ASN standard to the sample solution prior to extraction allows a quantitative and selective extraction of ASN. It is necessary to extract the ASA formulations first with diethyl ether in order to remove other ingredients used as auxiliaries, which would otherwise form emulsions with benzene and might also disturb the GLC determination. On the other hand, pure ASA samples could be dissolved directly in phosphate buffer solution and extracted with benzene. Cold solutions (24°) must be used in order to avoid the hydrolysis of ASN. The flame-ionization detector has to be cleaned from time to time, as it becomes contaminated with carbon particles when using benzene as the solvent. On examining a number of organic solvents, benzene was found to be the most suitable from the viewpoint of solubility (ASN) and specific gravity. It is not miscible with aqueous solutions, and forms an upper layer, thus facilitating injections without separation from the aqueous layer.

Fig. 4 shows a gas chromatogram for an ASN determination. Ten ASA formulations were examined; in six instances ASN was not detectable and in the other four samples the ASN content was between 0.002 and 0.08%. The standard deviation was found to be ± 0.004 and the coefficient of variation was $\pm 3.20\%$.

HPLC of ASN

This method is simple and needs no previous extraction of trace amounts of ASN from the sample. The increase in the methanol content of the mobile phase causes early elution of ASN. The separation is not sufficient for the quantitative

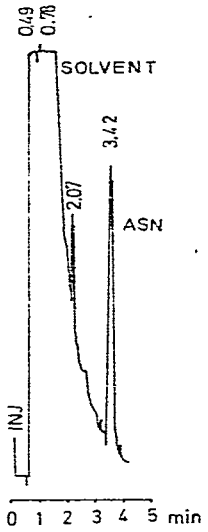


Fig. 4. Gas chromatographic determination of trace amounts of ASN in ASA. Column, 2 m × 4 mm I.D., coiled glass, packed with 3% OV-17 on Varaport 30 AW DMCS, 80–100 mesh; column temperature, 240°, isothermal; injector temperature, 280°; detector temperature, 300°; carrier gas, nitrogen, 30 ml/min.

evaluation of trace amounts of ASN. The resolution and retention are governed by the concentration of methanol in the mobile phase. It is well known from the viewpoint of column dynamics that a mobile phase of low viscosity should be chosen in order to achieve a rapid analysis at modest driving pressures. An increase in the water content of the mobile phase also enhanced its viscosity, but the desired resolution with an optimal analysis time was obtained only with a 45:55 mixture of water and methanol (Fig. 5).

Chromatographic packings with chemically bonded silicone phases provide excellent column efficiency and stability and eliminate the problems associated with the loss of partitioning phase from the support during the operation. Highly polar components such as water–methanol mixtures can be used to elute strongly retained sample components rapidly without effecting a change in the properties of the chromatographic column[22]. The use of a reversed-phase column coated with the chemically bonded C_{18} organic phase enabled the strongly retained trace amounts of ASN and ASSA to be separated from the bulk ASA.

The results of the ASN determination are presented in Table II; HPLC was not as sensitive as gas chromatography. The detection limit is about 0.01 %, and could be further increased as described for the SA determination. The standard deviation was found to be ± 0.075 and the coefficient of variation was $\pm 3.30\%$.

HPLC of ASSA

Optimal conditions were worked out for the simultaneous determination of ASSA and ASN impurities in ASA by HPLC. The separation was performed on a reversed-phase column isocratically with the mobile phase methanol (containing 1% glacial acetic acid)–water (48:52) with detection at 240 nm. Without the addition of

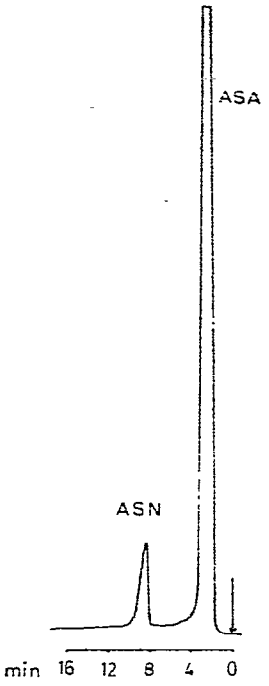


Fig. 5. HPLC separation of trace amounts of ASN from ASA on a reversed-phase column. Column, 0.25 m × 2.6 mm I.D., packed with silica gel SI-100 (10 μm, Merck), coated with chemically bonded organic phase C_{18}; mobile phase, water–methanol (45:55); pressure, 1200 p.s.i.; flow-rate, 1 ml/min; wavelength, 250 nm; temperature ambient; amount injected, 5 μl; recorder sensitivity, 5 mV; chart speed, 12 in./h.

TABLE II

DETERMINATION OF ASN IMPURITY IN ASA FORMULATIONS

Product	*ASN content (%)*	
	GLC	*HPLC*
A	Not detectable	Not detectable
D	0.081	0.06
E	Not detectable	Not detectable
F	0.003	Not detectable
G	0.01	0.01
H	Not detectable	Not detectable
I	Not detectable	Not detectable
K	0.002	0.01
L	Not detectable	Not detectable
M	0.038	0.031

acetic acid, a broad peak of poor resolution, not suitable for quantitative analysis, was obtained for ASSA and the analysis time was also prolonged. No separation of trace amounts of ASSA from ASA could be obtained on a Sil-X-I adsorption column with different solvent mixtures of diverse polarities (a, light petroleum–ethyl

acetate–glacial acetic acid, 77:20:3; b, ethyl acetate–glacial acetic acid, 97:3; c, cyclohexane–chloroform–methanol, 50:40:10).

The UV spectra of ASSA and ASN (Fig. 2) show a maximum in the region of 230 nm, but higher detection wavelengths were chosen in both instances on account of the absorption of some solvent impurities in the lower wavelength region where a zero baseline could not be obtained. The determination of trace amounts of ASN is to be preferred by the former method (Fig. 5) owing to the prolonged time of analysis (32 min) with the latter (Fig. 6).

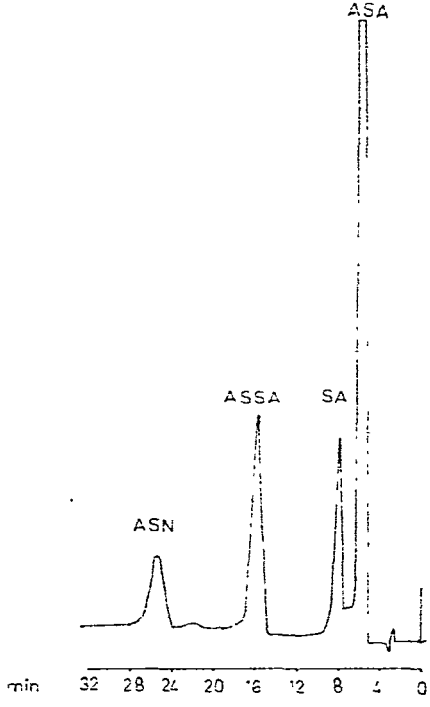


Fig. 6. Simultaneous separation of ASN and ASSA from ASA. Column, as in Fig. 5; mobile phase, water–methanol (containing 1% of acetic acid) (52:48); pressure, 1400 p.s.i.; flow-rate, 1 ml/min; wavelength, 240 nm; temperature, ambient; amount injected, 5 μl; recorder sensitivity, 5 mV; chart speed, 12 in./h.

In Fig. 6 a chromatogram is presented in which ASN, ASSA and partly SA are separated from ASA. The ASSA contents of four ASA formulations were determined quantitatively by the standard addition method. As for ASN, an amount equivalent to 500 mg of ASA was extracted with cold methanol, the volume made up to 5 ml with methanol and 5 μl of the solution were injected. Two samples showed ASSA contents of 0.028 and 0.05%, while in the other two formulations ASSA was not detectable.

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