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Note

Analysis of trace amounts of acetylsalicylic anhydride in acetylsalicylic acid

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Considerable interest has been generated in acetylsalicylic anhydride (ASN) as one of the significant impurities in acetylsalicylic acid (ASA): ASN may be a potential cause for allergic reactions to ASA[1,2]. The anhydride has been purported to be a highly immunogenic substance[1] in minute amounts and therefore a sensitive, specific analytical technique is required to determine quantitatively trace levels of ASN in ASA.

De Weck[1] described an extraction technique followed by thin-layer chromatography for the separation of ASN from ASA. His extraction technique has the disadvantage that 0.5 *N* sodium hydroxide must be added dropwise continuously over a period of 90 min to a phosphate buffer–benzene system, in order to maintain the desired pH. Bundgaard and Bundgaard[2] modified this extraction system so as to eliminate the dropwise addition of caustic solution. Their final analysis is more sensitive and is based on a spectrophotometric determination employing an α-benzamidocinnamate–pyridine reagent which they had to synthesize. Sethi[3] has modified the Bundgaard technique, and recently Ali[4] reported the use of gas and liquid chromatography for the determination of ASN in ASA.

The purpose of this paper is to describe a relatively simple, inexpensive, quantitative technique that appears to be specific for trace amounts of ASN in ASA.

EXPERIMENTAL

Materials

All solvents were analytical-reagent grade. The solvent systems employed for the chromatographic separations were methyl ethyl ketone–cyclohexane (50:50) and (70:30). Silica gel plates with fluorescent indicator (Silica Gel GF, Analtech, Newark, Del., U.S.A.; 10 × 20 cm and 20 × 20 cm, 250 μm) were activated at 105° for 1 h. The sample and standard solutions were spotted with the aid of an automatic spotting device (Analytical Instrument Specialities, Libertyville, Ill., U.S.A.).

The chromatograms were developed by ascending chromatography in a Gelman chromatography chamber (Gelman, Ann Arbor, Mich., U.S.A.), saturated with the vapor of freshly prepared solvents 15 min prior to development. A Chromata-Vue (Ultra-Violet Products, San Gabriel, Calif., U.S.A.) was used at 254 nm for the detection of spots after chromatography.

Procedure

Weigh 5.0 g of ASA into a 250-ml glass-stoppered erlenmeyer flask. Add 75 ml of ethyl acetate and stir with the aid of a magnetic stirring bar until the sample is dissolved. Add 125 ml of saturated sodium bicarbonate, cap and stir the two-phase system vigorously for 30 min. Place the contents into a 250-ml separatory funnel. Wash the erlenmeyer flask with 10 ml of ethyl acetate and transfer this to the separatory funnel. Discard the lower aqueous phase from the separatory funnel. Wash the ethyl acetate layer twice with 10-ml portions of water, discarding the aqueous layer each time.

Add about 2 g of anhydrous sodium sulfate (granular) to the ethyl acetate and shake. Pour off the resulting clear solution into a 150-ml beaker. (One also has the option of filtering the ethyl acetate solution into the beaker through filter paper). Wash the separatory funnel with 10 ml of ethyl acetate and transfer it to the beaker. Evaporate the solution to about 10 ml on the edge of a steam-bath and air evaporate the remainder to dryness in a hood without the aid of heat.

Add 5.0 ml of acetone to dissolve the faint residue and spot 100 μl onto the silica gel plate. Standards of ASN are prepared in acetone so that, for example, 20, 10, 5, 2 and 1 μg of ASN can be spotted along with the samples. Place the plate in the saturated chamber, let the solvent front move a distance of 10 cm from the origin, and remove the plate. Place the plate under a stream of cool air until nearly dry and then place the plate in the Chromata-Vue under UV light and compare the ASN spot of the sample to the standard ASN spots. The low-level spot (1 μg) tends to fade with time, therefore one should procede with the visual interpretation quickly.

RESULTS AND DISCUSSION

An important step in obtaining quantitative results for ASN in ASA is the order of addition of the extracting solvents. It is necessary to dissolve the ASA in the ethyl acetate before the addition of bicarbonate solution. This places the desired ASN into the organic solvent first (the more favorable phase because of its low solubility in water) and allows the ASA and any salicylic acid (SA) to be easily extracted out of the organic phase after the addition of the bicarbonate solution. Since Garrett[5] has demonstrated that the hydrolysis of ASN is 9 min at 26° and pH 8.0, then having the ASA intially in the ethyl acetate should decrease the possibility of hydrolysis of ASN by the sodium bicarbonate solution.

Recovery studies were performed by spiking samples of ASA with known amounts of ASN ranging from 0.003% to 0.05%. Recoveries were $\pm$ 0.001% ASN at the low levels and $\pm$ 0.005% ASN at the higher levels.

Visual observation under UV light showed one major spot to be present besides the ASN. This spot which has an approximate R_F value of 0.4 tails significantly and appears to coincide with ASA in its chromatographic profile and R_F value. The methyl ethyl ketone–cyclohexane solvent systems separate the ASN ($R_F = 0.6$) satisfactorily from the potential trace amounts of ASA ($R_F = 0.4$) and any possible SA ($R_F = 0.2$).

It appears from the chromatogram that a minute amount of ASA remains in the organic phase. This is quite conceivable considering the large sample of ASA used in the analysis. Since ASA can contribute to the oxazolone formation with the

Bundgaard reagent, one could possibly expect somewhat higher results for ASN in a sample of ASA. The technique of solvent extraction followed by thin-layer chromatography helps to increase the specificity of the method.

Several solvent systems as described by De Weck[1] were evaluated for the simultaneous separation of SA, ASA and ASN. However, the methyl ethyl ketone–cyclohexane mixtures provided the best results. Separation was slightly better with the 50:50 mixture than with the 70:30 mixture, although either ratio is quite acceptable.

Some concern was originally generated over the possibility of decomposition of ASN during the evaporation step under warm conditions. Chromatographic analysis of ASN with the warming (evaporation) technique was compared to a totally air-dried sample of ASN and to a control of ASN which did not undergo any evaporation step. The resulting chromatograms showed no salient difference in the chromatographic profiles or intensity of the spots. Likewise, it was established that standards can be used the next day as ASN prepared in acetone and kept for 24 h at room temperature showed no difference in intensity or chromatographic profile as compared to a freshly prepared standard.

Six different ASA samples, each of which was considered to have a low ASN content, were analyzed according to the procedure described in this article. Two samples were found to contain 0.002% ASN, one contained 0.005%, two contained 0.010% and the last one was found to contain 0.020% ASN. The results obtained are in the same order of magnitude as those published previously.[2–4]

One should keep in mind that the relative sensitivity of the method may be increased significantly. For example, one can easily reduce the amount of acetone to dissolve the residue from the evaporation step and/or increase the sample weight or volume of sample solution applied to the silica gel plate. Likewise, if only a limited amount of ASA is available, one should be able to decrease the sample weight as long as the ratio of the extracting solvents to sample size is maintained.

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