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GLC Determination of Purity of Schiff Bases Bakrine and Saddamine

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Abstract □ GLC methods were developed for the investigation of impurities in bakrine and saddamine. The method used for bakrine was unsuitable for saddamine since two possible saddamine impurities, benzylamine and salicylaldehyde, reacted very readily in solution to form saddamine, thus giving a false low value for the original concentration of these impurities. The method devised for saddamine involved silylation, which greatly reduced the possibility of saddamine formation from its precursors and also enabled the detection of another possible impurity, salicylic acid. The method described has an obvious application to the determination of other Schiff bases.

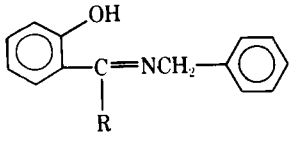
Keyphrases □ Bakrine and impurities—GLC analysis in prepared samples □ Saddamine and impurities—GLC analysis in prepared samples □ Anti-inflammatory agents, potential—bakrine and saddamine, GLC analysis in prepared samples

Bakrine, *N*-[1-(*o*-hydroxyphenyl)ethylidine]benzylamine (I), and saddamine, *N*-[*o*-hydroxyphenylmethylidine]benzylamine (II), are novel Schiff bases (1) with promising anti-inflammatory properties (2). These compounds are currently undergoing clinical trials in Iraq.

During the development of a method for the determination of the purity of bakrine and saddamine samples, spectrophotometric and TLC procedures were shown to lack specificity; GLC was the method of choice because of its speed, sensitivity, and specificity. However, although a solution of bakrine containing expected impurities could be analyzed satisfactorily by direct injection onto the GLC column, a similar procedure for saddamine gave inconsistent results. Therefore, the possibility that saddamine could be reformed from any salicylaldehyde and benzylamine present as impurities in the final product was investigated.

EXPERIMENTAL

Reagents and Materials—Bakrine[1], saddamine[1], and bis(trimeth-



I: R = CH_3
II: R = H

[1] Maybridge Chemical Co., Cornwall, England.

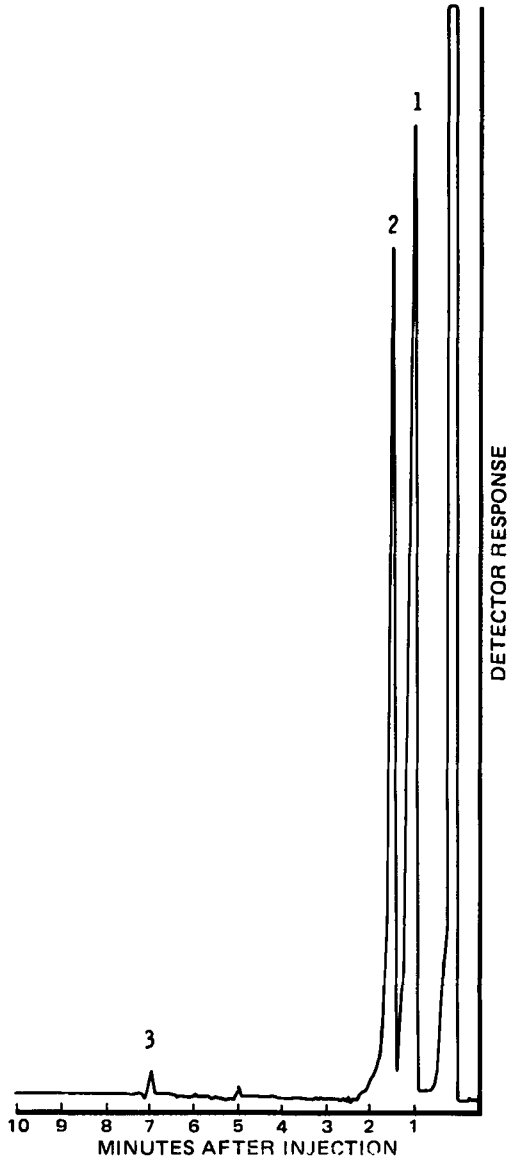


Figure 1—*Gas chromatogram of benzylamine (peak 1) and 2-hydroxyacetophenone (peak 2) in 1,2-dichloroethane. Very little bakrine (peak 3) was formed.*

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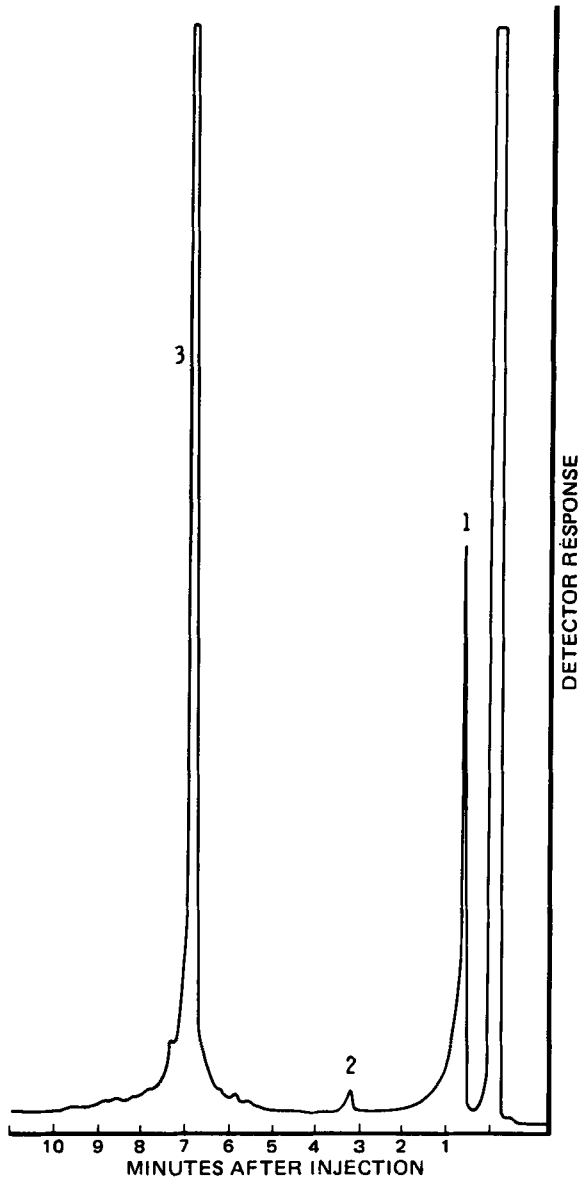

Figure 2—*Gas chromatogram of benzylamine (peak 1) and salicylaldehyde (peak 2) in 1,2-dichloroethane. Saddamine (peak 3) formation was almost quantitative.*

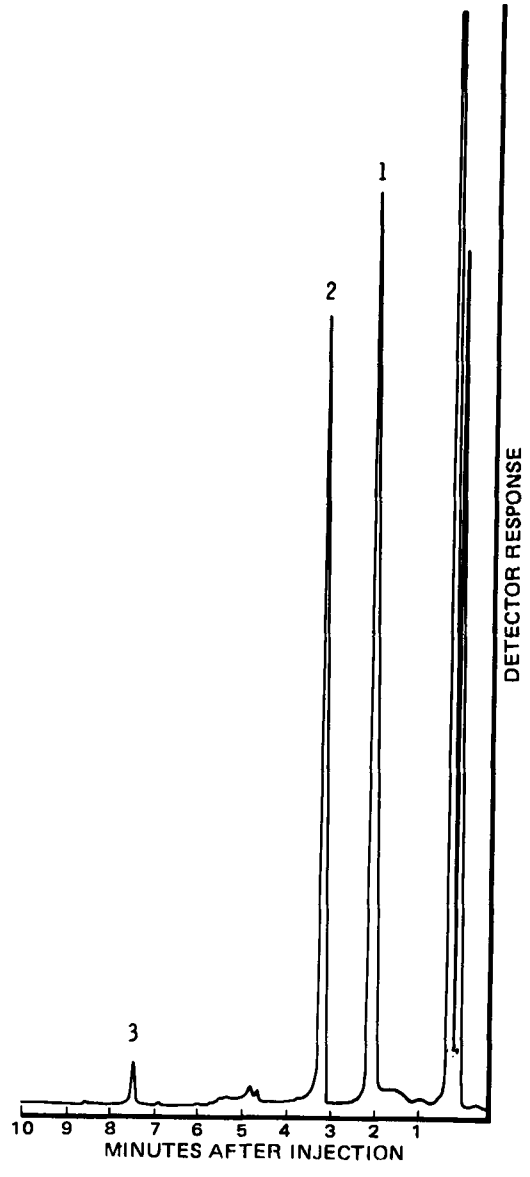

Figure 3—*Gas chromatogram of derivatized benzylamine (peak 1) and salicylaldehyde (peak 2). The formation of saddamine (peak 3), in this case derivatized, was greatly reduced.*

ylsilyl)trifluoroacetamide[2] were used as supplied. All other solvents and reagents were analytical reagent grade. Screw-capped 1-ml tapered reaction vials, fitted with polytef-faced rubber seals[3], were used for silylation.

Apparatus—A gas chromatograph[4] was equipped with a flame-ionization detector and a 2-m × 2.5-mm i.d. stainless steel column packed with 5% OV-1 on 100–120-mesh Gas Chrom Q[5]. The column was conditioned before use at 275° for 24 hr with the carrier gas (nitrogen) at a flow rate of 35 ml/min. The chromatograph was operated under the following conditions: column and injection port temperature, 275°; detector temperature, 275°; carrier gas flow, 30 ml/min; air flow, 300 ml/min; and hydrogen flow, 40 ml/min. The oven temperature was initially 130° for 2 min and was then increased to 260° at a rate of 20°/min.

Silylation Procedure—Trimethylsilyl derivatives were prepared by the addition of 250 μl of silylating reagent to 5 mg of the test compounds in separate reaction vials. The vials were capped immediately and left for 2 hr at room temperature (20°). The silylation reaction went to completion after 2 hr for all compounds tested, and the derivatives were stable for at least 24 hr when kept in the sealed vials.

[2] Regisil, Regis Chemical Co., Chicago, Ill.
[3] Regis Chemical Co., Chicago, Ill.
[4] Perkin-Elmer model F33.
[5] Phase Separations, Clwyd, Wales.

For the chromatography of derivatives, suitable volumes of solutions of the individual silylated compounds were mixed and diluted with silylating reagent to give a final concentration of 2 mg/ml for each component in the mixture. Aliquots of 1 μl were injected onto the column.

RESULTS AND DISCUSSION

GLC determination of the purities of bakrine and saddamine was first attempted by separate injections of 1,2-dichloroethane solutions of the two compounds and mixtures of their carbonyl and amino precursors, respectively. This procedure was satisfactory for bakrine; as shown in Fig. 1, a mixture of benzylamine (0.1 ml) and 2-hydroxyacetophenone (0.1 ml) in 1,2-dichloroethane (10 ml) reacted to produce less than 1% of the theoretical yield of bakrine after 2 hr at room temperature. With benzylamine and salicylaldehyde under the same conditions, however, almost quantitative conversion to saddamine took place (Fig. 2). If these compounds had been present in the original saddamine, the purity determination would have been invalidated.

Since it was necessary to eliminate this source of error, trimethylsilyl derivatives of the two saddamine precursors were used to reduce their reactivity. Such derivatization had a further advantage in that salicylic acid, another potential impurity that cannot be chromatographed directly, may be determined successfully under these conditions (3–5). Chromatography of a mixture of the trimethylsilyl derivatives of benzylamine and salicylaldehyde left at room temperature for 2 hr showed

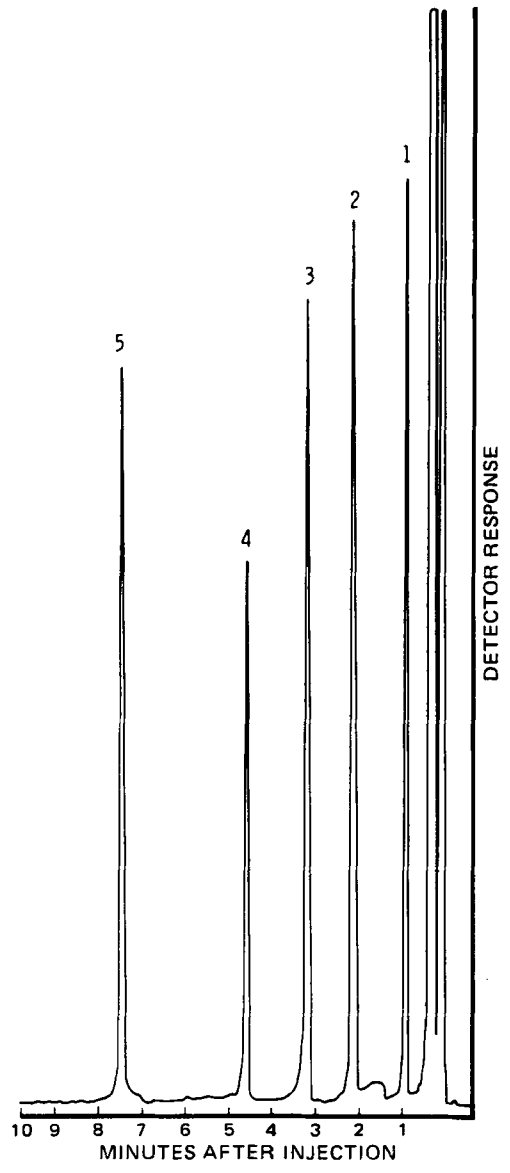

Figure 4—*Gas chromatogram of derivatized phenol (peak 1), benzylamine (peak 2), salicylaldehyde (peak 3), salicylic acid (peak 4), and saddamine (peak 5), each present at a concentration of 2 mg/ml of silylating solution.*

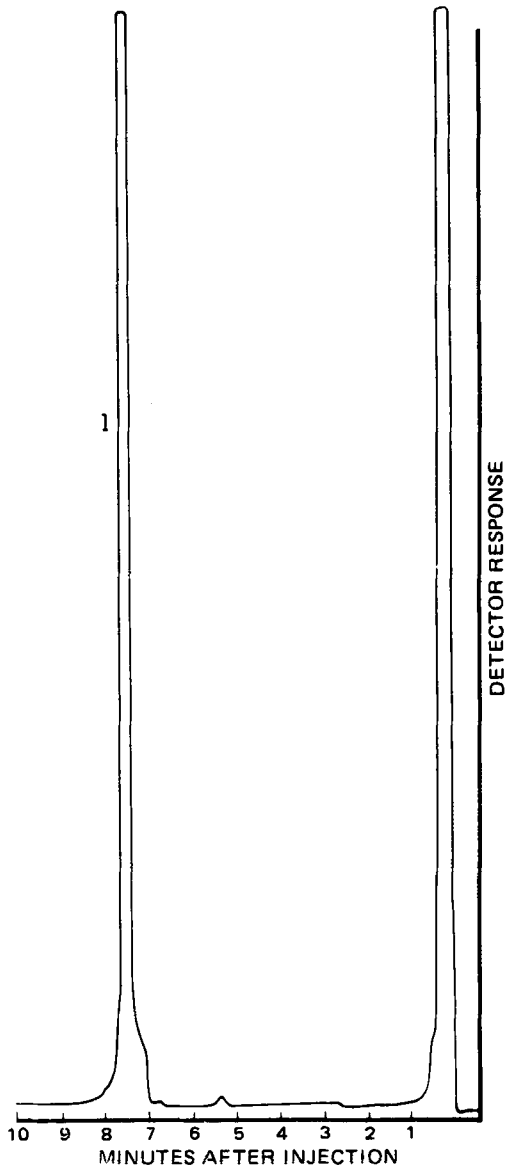

Figure 5—*Typical gas chromatogram of saddamine (peak 1) purity determination.*

that formation of the trimethylsilyl derivative of saddamine amounted to less than 5% of the total theoretical yield (Fig. 3). Thus, with this method, benzylamine and salicylaldehyde would be easily detected should they occur together as saddamine impurities.

Chromatography of a mixture of the trimethylsilyl derivatives of phenol, benzylamine, salicylaldehyde, salicylic acid, and saddamine (Fig. 4) indicated that there was good separation of the saddamine derivative from the derivatives of the potential impurities. The impurities present in many samples of saddamine were investigated using this method, and the chromatogram shown in Fig. 5 is typical of those obtained after on-column injection of 1 μl of a 20-mg/ml solution of the trimethylsilyl derivative of saddamine. Each potential impurity shown in Fig. 4 was absent down to a level of less than 0.5%.

The described method has an obvious application to the determination of other Schiff bases.

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