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Transesterification: an analytical and formulation problem

W. J. IRWIN*, Q. N. MASUDA and A. LI WAN PO

Pharmaceutics Research Group, Division of Pharmaceutics and Pharmaceutical Chemistry, Department of Pharmacy, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET, UK

Abstract: A range of esters including salicylates, nicotinates and parabens have been shown to undergo reversible, base-catalysed transesterification in hydroalcoholic solutions. In non-alcoholic solution phenyl salicylate undergoes a concentration-dependent oligomerization which yields salsalate and other products. The transesterification reactions also occur in products formulated for topical use, which have vehicles based upon alcohol, glycol or glycol polymers. Without recognition, such reactions may compromise stability assessments, pharmaceutical integrity and delivery profiles.

Keywords: *Transesterification; salicylates; nicotinates; high-performance liquid chromatography; topical formulations; stability; parabens.*

Introduction

When esters of carboxylic acids, dissolved in an alcohol which does not correspond to the ester radical, are heated with traces of an acid or base interchange of alcohol residues may be observed and a new ester can be isolated. This process is known as transesterification and has many synthetic uses [1]. These procedures frequently involve strong conditions and the pharmaceutical importance of such reactions under the milder conditions of analytical or formulation work have not been stated. The use of hydroalcoholic solutions for physico-chemical and analytical work and the availability of alcoholic excipients such as propylene glycol and the polyethylene glycols, has led to work that has shown that many transesterification reactions proceed under mild conditions. These facile reactions threaten the integrity of pharmaceutical formulations, and analytical work which is not based upon highly specific assays may be compromised.

Examples of transesterification which are of pharmaceutical importance include the intramolecular migration of the 17-esters of hydrocortisone or betamethasone to the less active 21-isomers [2, 3]. Although this conversion is accelerated by inappropriate formulations, pre-formulation stability studies enable these problems to be readily identified. A more insidious transesterification is that involving salicylate esters used as topical analgesics [4] where the alcoholic component present as the vehicle or the solvent

*To whom correspondence should be addressed.

interchanges rapidly with that of the drug to produce a second ester. As the substantivity and percutaneous absorption of salicylates is markedly dependent upon the ester function [5] such transformations may lead to a significant change in the activity profile. Stability profiles, too, may be erroneous if determined in hydroalcoholic media. In alkaline 4% ethanol, for example, phenyl salicylate was shown to have a half-life of 9.4 min when monitored by HPLC [6]. This very short half-life can be contrasted with an estimate of 40 min based upon a simple UV assay which could not detect the rapid formation of the more stable ethyl ester. Other solvent–vehicle interactions with medicaments recently reported include the increased melting point of aminophylline suppositories through reaction of ethylenediamine with the fatty base [7] and also the presence of indomethacin and *p*-chlorobenzoic acid esters of polyethylene glycols in indomethacin suppositories [8, 9]. As a timely alert to these potential problems examples are presented of some transesterification reactions, which show the rapid rate of conversion exhibited under certain conditions.

Experimental

Chromatography

Analyses were performed using a high-performance liquid chromatograph (HPLC) constructed from an Altex 100A dual-reciprocating, constant-flow solvent-metering pump, a Rheodyne 7120 injection valve fitted with a 20 μl loop and a Pye LC3 variable wavelength ultraviolet monitor equipped with an 8 μl flow cell and set at 235 nm; the sensitivity was 0.16–0.32 a.u.f.s. A Shandon 100 × 4.6 mm i.d. stainless steel column was packed with 5 μm ODS-Hypersil reversed-phase material. The pre-filtered (0.45 μm) mobile phases were: for methyl salicylate and methyl 2-methoxybenzoate, CH_3CN–H_2O–H_3PO_4 (50:49:1, v/v/v), pH = 1.85; for methyl nicotinate, CH_3CN–H_2O–H_3PO_4(0.17%, m/v)–NH_3(0.17%, m/v) (40:59.45:0.05:0.5, v/v/v/v), pH = 6.8; for methyl 4-hydroxybenzoate, CH_3CN–H_2O–H_3PO_4 (35:64:1, v/v/v), pH = 1.7; for phenyl salicylate, CH_3CN–H_2O–H_3PO_4 (54:45:1, v/v/v). pH = 1.88; and for salol aqueous cream, CH_3CN–H_2O–H_3PO_4 (50:49.5:0.5, v/v/v), pH = 2.0 and CH_3CN–H_2O–H_3PO_4, (25:74.9:0.1, v/v/v), pH = 2.0. The flow-rate was 1 ml min^{-1}.

Methods

Transesterification and hydrolysis of methyl nicotinate

Double-strength Britton–Robinson buffer solutions were prepared in the pH range 1.81–11.2 [10] so that a 1:1 (v/v) dilution gave the published compositions with an ionic strength of 0.5 M. The appropriate buffer solution (50 ml) and an equimolar mixture of methanol–ethanol (32:46, m/m) (50 ml) were mixed and equilibrated at 50°C. A freshly prepared stock solution of methyl nicotinate (5 mM) in water (2 ml) was added to each reaction vessel and the temperature was maintained at 50°C. Concentrations of the reactant (methyl nicotinate), the intermediate (ethyl nicotinate) and the product (nicotinic acid) were determined by HPLC immediately and at suitable time intervals. Aliquots (2 ml) were withdrawn and quenched by addition to a cooled solution (2 ml) of the internal standard, ethyl 4-hydroxybenzoate in 0.01 M HCl (0.0075%). A 20-μl aliquot was injected onto the column. Concentrations were calculated by interpolation from a calibration curve over the concentration range 0.02–0.2 mM for the ethyl ester and 0.1–1 mM for other components [11, 12].

Transesterification and hydrolysis of methyl nicotinate (1 mM) were monitored in 50% ethanol containing 0.01, 0.001, and 0.0005 M NaOH.

Transesterification of methyl salicylate, methyl 2-methoxybenzoate and methyl 4-hydroxybenzoate

Reaction solvents were prepared by adding an equimolar mixture of methanol–ethanol (32:46, m/m) (25 ml) to 1 M NaOH (25 ml) and equilibrating at 80°C for methyl 4-hydroxybenzoate and at 30°C for the other esters. At zero time the ester stock solution (50 mM, 1 ml) in methanol–ethanol (32:46, m/m) was added to each reaction vessel. The concentrations of reactant (methyl ester), transesterified intermediate (ethyl ester) and product (acid) were determined by HPLC. Aliquots (2 ml) were withdrawn at appropriate time intervals and were quenched by addition to the cooled internal standard solution (2 ml) of propyl 4-hydroxybenzoate in 0.5 M HCl (0.008%) for the salicylate and 4-hydroxybenzoate assays or butyl 4-hydroxybenzoate in 0.5 M HCl (0.009%) for the methyl 2-methoxybenzoate assays. A 20-μl aliquot was injected onto the column. Concentrations were calculated by interpolation from a calibration curve over the concentration range 0.1–1 mM for each component.

Hydrolysis of phenyl salicylate: formation of salicyl salicylate

A stock solution of phenyl salicylate (10 mM) in acetonitrile (1 ml), stored in an ice-bath, was added to 0.01 M NaOH in 50% (v/v) aqueous acetonitrile at 37°C and aliquots were withdrawn periodically for HPLC analysis. Analogous experiments with phenyl salicylate (0.1, 0.2 and 0.4 mM) in 50% (v/v) acetonitrile–0.05 M borate buffer (pH 7.55) in the temperature range 37–50°C were conducted similarly.

Stability of methyl salicylate gels

A series of Carbopol gels of different pH values were prepared by dissolving methyl salicylate (0.25 g) in ethylene glycol (40 g) and water (4 ml). Carbopol 934 (1 g) was dispersed by stirring and the gel was formed by the addition of 5 ml of an appropriate concentration of sodium hydroxide (0.4–2 M). The pH values of the gels, measured on dilutions of 1 g of gel with water (10 ml), were 4.92, 5.28, 5.45, 6.66 and 8.06. Gels were stored at 37°C for 72 h. For analysis the gel (1 g) and potassium chloride (300 mg) were dispersed in acetonitrile (5 ml). The mixture was centrifuged at 6000 r.p.m., 1 ml of the supernatant solution was diluted 1 in 10 with water and a 20 μl aliquot was analysed by HPLC.

Stability of salol aqueous cream

A freshly prepared batch of cream containing stearic acid (16 g), wool fat (2 g), propylene glycol (5 g), phenyl salicylate (10 g), triethanolamine (2 g) and water (to 100 g) was stored at 37°C. At intervals a sample (1 g) and potassium chloride (300 mg) were dispersed in acetonitrile (5 ml). The mixture was centrifuged at 6000 r.p.m., 1 ml of the clear solution was diluted to 100 ml with 50% (v/v) aqueous acetonitrile and a 20 μl aliquot was analysed by HPLC.

Transesterification with 4-dimethylaminopyridine

Methyl nicotinate (1 mM) or methyl salicylate (1 mM) were dissolved in 50% (v/v) aqueous ethanol containing 0.01 or 0.02 M 4-dimethylaminopyridine at 37°C. The transesterification and hydrolysis reactions were monitored by HPLC as described above.

Results and Discussion

Transesterification in solution

Figure 1 records the HPLC trace of methyl nicotinate dissolved in 50% alkaline aqueous ethanol at 37°C. At zero time methyl nicotinate (a) and the internal standard (d) were the only components present but after 1 min significant degradation in solutions containing 0.0005 M NaOH was observed and ethyl nicotinate (b) and nicotinic acid (c) were readily detected. The structures of these products were confirmed by isolation and spectroscopic analysis. In those solutions containing higher amounts of sodium hydroxide, methyl nicotinate had totally disappeared within 5 min although traces of the ethyl ester were detectable for up to 30 min (0.001 M NaOH) or 10 min (0.01 M NaOH).

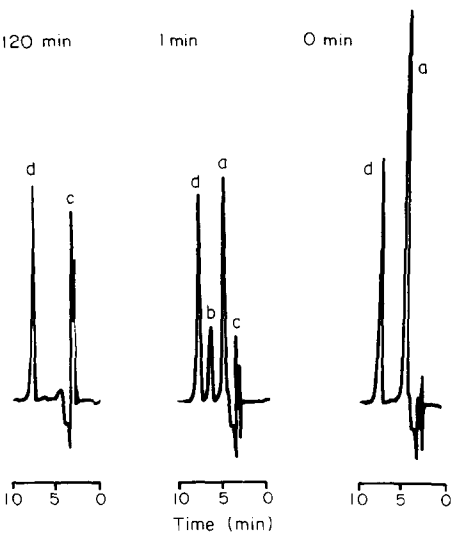
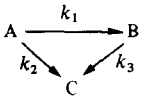


Figure 1
High-performance liquid chromatograms of methyl nicotinate in 50% (v/v) aqueous ethanol containing 0.005 M NaOH at 37°C. a, Methyl nicotinate; b, ethyl nicotinate; c, nicotinic acid; d, ethyl 4-hydroxybenzoate, internal standard.

These reactions follow the model:

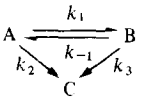


(1)

where A, B and C represent the methyl ester, ethyl ester and parent acid respectively; k_2 and k_3 are the first order hydrolysis rate constants and k_1 is the first order transesterification rate constant.

The rate constants obtained by non-linear least squares regression [4, 6] were $k_1 = 0.0263\ \text{min}^{-1}$, $k_2 = 0.0125\ \text{min}^{-1}$ and $k_3 = 0.00143\ \text{min}^{-1}$ for the reaction with 0.0005 M NaOH and these values confirm the rapid degradation.

When the solvent also includes methanol, reversible transesterification is observed and the appropriate model is now:



(2)

where k_1 and k_{-1} are the forward and reverse transesterification rate constants.

Reaction profiles similar to that in Fig. 2 were obtained and the individual rate constants were again evaluated using non-linear least squares regression [4, 6]. Figure 3 records these data as a logarithmic function of pH. The plots are linear and show that under the experimental conditions transesterification rates exceed the hydrolysis rates. When water is eliminated from the reaction medium only transesterification is observed.

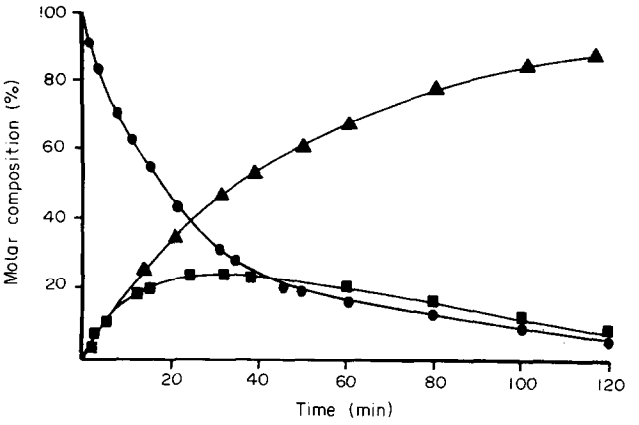


Figure 2
Concentration–time graph for degradation of methyl nicotinate in 50% (v/v) aqueous methanol–ethanol (32:46, m/m) (pH 11.40) ● Methyl nicotinate; ■ ethyl nicotinate; ▲ nicotinic acid, $k_1 = 0.0257\ min^{-1}$, $k_{-1} = 0.0285\ min^{-1}$, $k_2 = 0.0232\ min^{-1}$, $k_3 = 0.0115\ min^{-1}$.

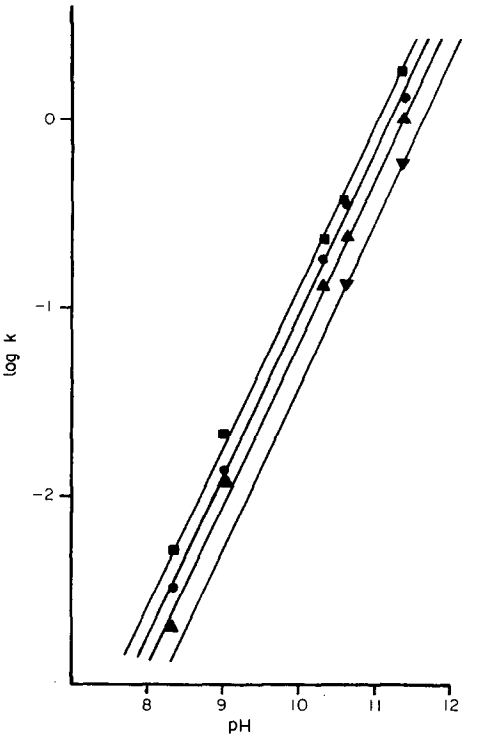


Figure 3
Transesterification and hydrolysis of methyl nicotinate in 50% (v/v) aqueous ethanol (pH = 11.40). Graph of log k against pH. (●, k_1; ■, k_{-1}; ▲, k_2; ▼, k_3).

Little reaction is observed in the pH range 2.5–7 and the increase in rate of reaction with pH indicates that the most probable mechanism involves direct attack of hydroxide and alkoxide ions on the ester function. This is in contrast to the reactivity of salicylate esters in which a concerted attack of solvent molecules upon the salicylate anion is the main reaction under weakly alkaline conditions. Both salicylate and nicotinate also exhibited transesterification when 4-dimethylaminopyridine [13], rather than hydroxide, was used as the basic catalyst.

Transesterification reactions are also displayed by other esters. Methyl salicylate, methyl 2-methoxybenzoate and methyl 4-hydroxybenzoate in the mixed alcohol solvent all showed the presence of the transesterified product and Table 1 presents the rate constants derived from these experiments. Those from methyl 4-hydroxybenzoate were obtained at 80°C to allow significant reaction to be observed. This resistance to base degradation is due to ionization of the phenolic residue which reduces the reactivity of the ester function towards nucleophilic attack by hydroxide or alkoxide ions. For salicylate esters this ionization makes available a concerted transesterification–hydrolysis pathway involving solvent molecules whereas for methyl 2-methoxybenzoate, no initial ionization is possible and degradation rates are significantly faster.

Table 1
Degradation rate constants for transesterification and hydrolysis of methyl salicylate, methyl 2-methoxybenzoate and methyl 4-hydroxybenzoate in a mixture of equal volumes of methanol–ethanol (32:46, m/m) and 1 M NaOH.

Compound	Temperature (°C)	Rate constant ($min^{-1} \times 10^3$) k_1	k_{-1}	k_2	k_3
Methyl salicylate	30	9.46	29.5	5.73	0.496
Methyl 2-methoxybenzoate	30	29.9	43.9	155	148
Methyl 4-hydroxybenzoate	80	22.46	2.004	50.55	67.05

Transesterification in formulations

To examine the effect of formulation on transesterification a series of semi-aqueous gels containing methyl salicylate was prepared. Gels made with ethylene glycol rather than propylene glycol were used to evaluate stability since reference standards of glycol salicylate, the potential product, are readily available. By varying the amount of sodium hydroxide added to the Carbopol base a range of gels with pH values of 4.92–8.06 were obtained. Chromatograms of these gels after storage at 37°C for 72 h are shown in Fig. 4 and clearly reveal the potential transesterification problem. At pH 4.92 no degradation is evident but at all higher pH levels degradation is observed. At pH 5.45 and pH 5.28 only traces of glycol salicylate (b) are apparent but as the medium becomes sufficiently alkaline to support higher levels of salicylate anion the transesterification reaction increases in importance and at pH 8.06 the glycol ester is the major component of the formulation. Competitive hydrolysis due to the small amount of water in the formulations is also indicated by the appearance of salicylic acid (c), although this is not the major degradation pathway.

Surgical spirit is a product which contains methyl salicylate, diethyl phthalate and castor oil in an ethanolic solution. Transesterification is possible in this formulation with production of ethyl salicylate. Of five samples examined, a freshly purchased sample showed only the methyl ester but four older preparations indeed showed traces of ethyl

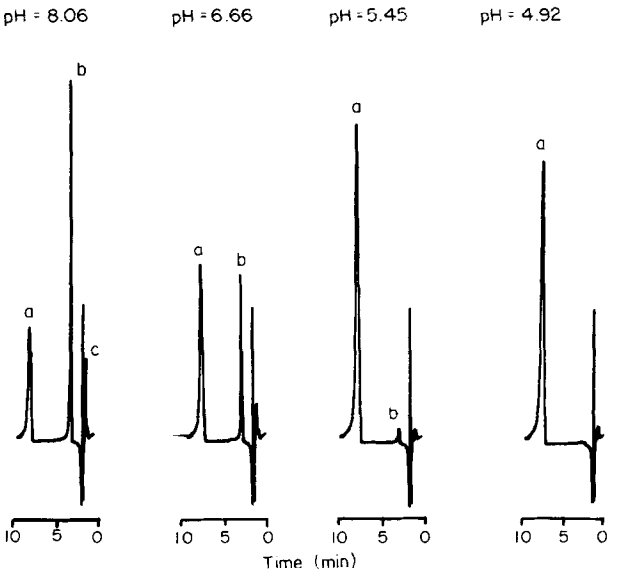


Figure 4
High-performance liquid chromatograms of methyl salicylate gels of different pH values. a, Methyl salicylate; b, glycol salicylate; c, salicylic acid.

salicylate in solution. Products containing methyl salicylate with isopropyl alcohol [14] and phenyl and methyl salicylate with propylene glycol [15] also undergo transesterification reactions on storage.

The transesterification reactions discussed so far have involved reaction with the solvent or vehicle. It would appear possible that suppression of unwanted reactions merely involves the selection of an inert vehicle. This is not necessarily true. For example, phenyl salicylate undergoes rapid transesterification in alcoholic solution [6] but the degradation of solutions in alkaline aqueous acetonitrile is not limited to the expected hydrolysis to salicylic acid and phenol. Figure 5 shows that in addition to the expected products a 0.2 mM phenyl salicylate solution yielded a fourth peak. Formation of this component was concentration dependent; the peak was not produced in 0.1 mM phenyl salicylate solution but was enhanced, together with a further new peak, in 0.4 mM solutions. This product has been identified as salicyl salicylate (salsalate) produced by dimerization of phenyl salicylate (Scheme 1), possibly together with the trimer. The disappearance of phenyl salicylate under the conditions which yield dimer as the only oligomeric product is described by the system.

$$\begin{array}{ccc} & \xrightarrow{k_2} & \\ A + A & & B \\ & \searrow^{k_1} \quad \swarrow_{k_3} & \\ & C & \end{array} \qquad (3)$$

where A represents phenyl salicylate, B salicyl salicylate and C salicylic acid; k_1 and k_3 are first-order hydrolysis rate constants and k_2 is the second-order dimerization rate constant. The concentration (A_t) of species A at any time t is given by:

$$A_t = \frac{A_o.k_1.\exp(-k_1 t)}{k_1 + A_o.k_2 - A_o.k_2.\exp(-k_1 t)} \qquad (4)$$

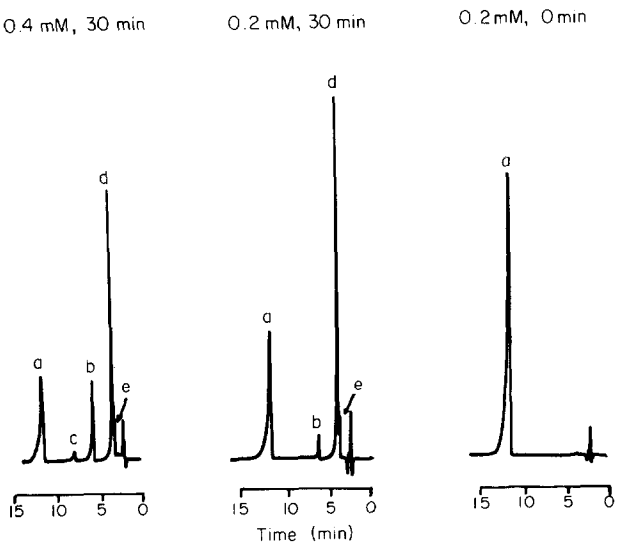
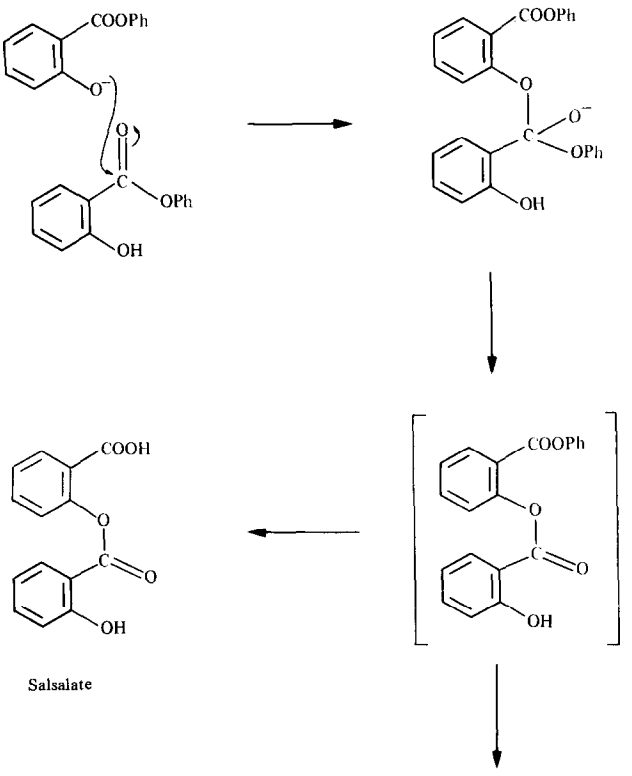


Figure 5
High-performance liquid chromatograms of phenyl salicylate in 0.01 M NaOH in 50% (v/v) aqueous acetonitrile. a, Phenyl salicylate; b, salicyl salicylate (salsalate); c, possibly salicyl salicyl salicylate; d, salicylic acid; e, phenol.



Scheme 1
Formation of salsalate from phenyl salicylate

Non-linear least squares regression of the measured profile using this model provides an estimate for k_1 of 0.0160 min^{-1}. When $k_1 \gg A_0.k_2$ the k_2 terms in the denominator of equation (4) may be neglected. In this experiment the first-order rate constant considerably exceeds that of the second-order dimerization rate constant and the discrepancy between the exact approach and the estimate obtained using the limiting first-order model is small (k_1 = 0.0164 min^{-1}).

This type of reaction parallels those of aspirin observed in solid-state degradation [16–20] which yield products with immunogenic properties [21, 22]. Were such transformations to occur in topical formulations allergic reactions to the preparation might be expected. Such reports have been made concerning topical phenyl salicylate preparations used as sunscreens [23]. Salol aqueous cream is a product which contains phenyl salicylate in a cream base with 5% of propylene glycol. This clearly has some potential for degradation by transesterification or dimerization although the biphasic

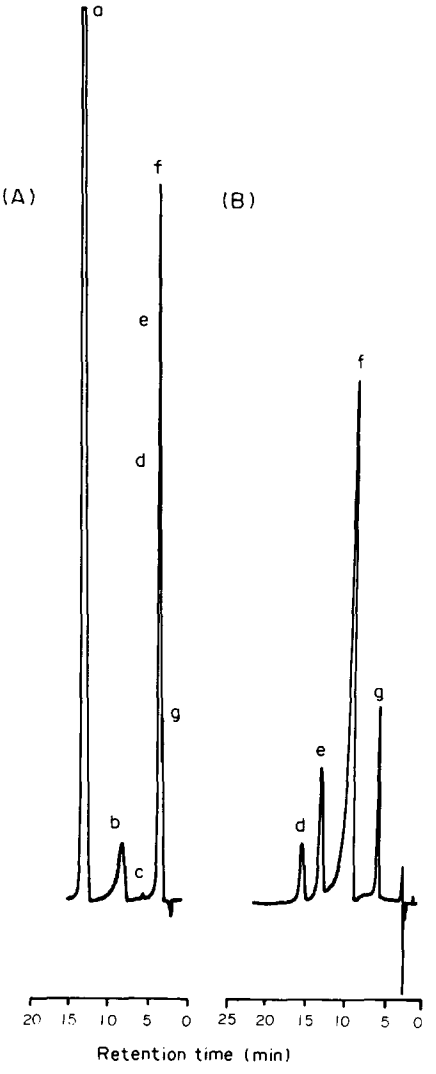


Figure 6
High-performance liquid chromatogram of salol aqueous cream stored at 37°C for 37 days. A, With a mobile phase of 50% (v/v) acetonitrile (pH = 2.0). B, With a mobile phase of 25% (v/v) acetonitrile (pH = 2.0). a, Phenyl salicylate; b, triethanolamine-salicylate product, c, salicyl salicylate (salsalate); d, 1-hydroxymethylethyl salicylate; e, 2-hydroxypropyl salicylate; f, salicylic acid; g, phenol.

formulation may provide a considerable stabilizing influence. Figure 6 records the HPLC trace of the cream after storage for 37 days at 37°C. Considerable degradation is evident and the transesterified propylene glycol products are readily identified. A rather broad peak is also observed in the trace. This product is formed when phenyl salicylate and triethanolamine, a component of the cream, are stored together and is probably a further transesterified component. Additionally, traces of salsalate are also detected. This indicates that dimerization, and possibly oligomerization, are potential degradation pathways in this formulation and may lead to adverse activity profiles.

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