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Comparative study of lactic acid and polylactides using static headspace, gas chromatography and high-performance liquid chromatography

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Abstract

This work investigates the use of analytical methodology to determine lactic acids and/or polylactides. This paper reports three methods that have been successfully developed. Firstly, static headspace gas chromatography (GC) was investigated. The results show precision values of 4–5% RSD and linearity correlation coefficients of between 0.990 and 0.999 in the concentration range 300–700 μg/ml lactic acid. The method is specific for lactic acid. Secondly, an on-column GC method is presented which minimises sample preparation and separation of lactic acid from the polylactides. Finally, an ion-exclusion high-performance liquid chromatography (HPLC) method was developed. The instrumental precision was 0.1%, and linearity correlation coefficients >0.9999 were obtained in the concentration range between 50 and 200 μg/ml lactic acid. A basic hydrolysis is used to convert the polylactides to lactic acid. Collectively, these methods form a successful approach for the determination of lactic acid in consumer healthcare products. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Headspace analysis; Lactic acid; Polylactides; Sodium lactate

1. Introduction

The determination of lactic acid in consumer healthcare products is complicated due to varied formulations, often involving difficult matrices. The British Phamacopeia [1] and United States Phamacopeia [2] monographs on lactic acid state that the acceptable purity in pharmaceutical formulations is between 88 and 92% and between 85 and 90%, respectively.

The main process-related impurities of lactic acid are the self-condensed linear polylactides, e.g. lac-

toyl-lactic acid [3]. These polylactides are caused by self-polymerisation and are present in equilibrium with lactic acid. The polylactide concentrations are affected by temperature, pH and lactic acid concentration. The dimer (lactoyl-lactic acid) is the most abundant followed by the trimer

$$CH_3CH(OH)CO_2H \underset{}{\overset{H^+/H_2O}{\rightleftarrows}} CH_3CH(OH)COOCH(CH_3)CO_2H + H_2O$$

lactic acid   lactoyl lactic acid (dimer)

Lactic acid has been analysed in various matrices by various techniques. High-performance liquid chromatography (HPLC) has been used to analyse lactic acid in dermatological products by Cheng and Gadde

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[4]. They described two approaches to determining lactic acid which both hydrolyse the polylactides to lactic acid for both standard and sample solutions. After hydrolysis two separative methods were reported. Firstly, reversed-phase liquid chromatography (RPLC) with ion-pairing with a reported analysis time of about 40 min and, secondly, using an amine column for ion exchange.

The use of ion-exclusion HPLC for the analysis of lactic acid has been reported by Andersson et al. [5] and Ashoor et al. [6], where the stationary phase is polystyrene–divinylbenzene (PS–DVB). The field of application of Andersson et al. was food and drinks and for Ashoor et al. biofluids. Hitherto its application for analysis of lactic acid in consumer healthcare products has not been reported.

Gas chromatography (GC) has been used for lactic acid analysis with most reported methods [7–9] involving derivatisation typically using t-butyldimethylsilyl or oxidation to acetaldehyde. Derivatisation is often a lengthy exercise and may not be appropriate for a high throughput laboratory. Heitfuss et al. [10] developed a method using static headspace GC with methylation of lactic acid in the headspace vial followed by automatic injection onto the column. The method was applied to microbiological preparations but here the matrices were simple with relatively few potential interfering compounds or compounds which can compete in the methylation. The method has been further developed in this work so as to apply it to consumer healthcare products.

A direct injection method for the analysis of lactic acid has been reported using on-column injection and water–oxalic acid as solvent with limited success [11].

This paper investigates the application of three methods that can be used to analyse either lactic acid, sodium lactate or polylactides in consumer healthcare products. The first of these is a HPLC method utilising hydrolysis for sample preparation as detailed by Cheng and Gadde [4] followed by separation on a PS–DVB column.

The second method uses headspace GC based on Heitfuss et al. [10], however two coupled columns of a non-polar phase and a medium polar phase are used to separate methylated lactic acid. The methylation conditions were 120°C for 20 min. However, under the methylation conditions the polylactides present are converted back to lactic acid.

The final method is a development of McCalley's [11] method using an on-column GC method with methanol as sample solvent and separating lactic acid from polylactides. Mass spectrometry was used for identification for both the headspace and on-column GC methods.

2. Experimental

2.1. Chemicals and reagents

L-Lactic acid (98%) was obtained from Sigma (Poole, UK). This was degraded at 25°C for 1 month. L-Lactic acid (>99%) and sodium lactate (>99%) were purchased from Fluka (Gillingham, UK). DL-Lactic acid (88–92% purity) was supplied by Puric Biochem (Birmingham, UK).

Methanol, sodium hydrogensulphate ($NaHSO_4$) and orthophosphoric acid were analytical-reagent grade and 0.05 M sulphuric acid (H_2SO_4) and 1 M sodium hydroxide (NaOH) 'Solutrate' standards were supplied by Fisher (Loughborough, UK). Oxalic acid (analytical-reagent grade) was supplied by Merck (Poole, UK). Carrier gas for GC was helium (99.9999% purity) supplied by Air Products (Cheshire, UK).

Two different consumer healthcare experimental lactic acid placebo formulations (without lactic acid) prepared in the laboratory were used as samples.

2.2. Apparatus and conditions

2.2.1. HPLC

Chromatography was performed using a Shimadzu HPLC system (Shimadzu, Japan); a Model SCL-6B and SIL-6B auto injector, an LC 9A pump and a SPP-6AV UV detector (214 nm) were used. The column was a PS–DVB divinylbenzene organic acids phase column (Chrompack, Middelburg, The Netherlands), 30 cm×0.65 cm I.D., 9 μm particle size. The data were analysed using the PeakPro vax data handling system (Beckman, Fullerton, CA, USA). The mobile phase was 0.05 mM sulphuric acid. The flow-rate was 0.7 ml/min and the injection volume was 50 μl.

2.2.2. Headspace gas chromatography

Headspace gas chromatography was performed using a Perkin-Elmer (Norwalk, CT, USA) autosystem gas chromatograph fitted with a HS4O headspace analyser and a flame ionization detector. The column system used was a CP-Sil 5CB (10 m×0.32 mm I.D., 1.0 μm d_f, methyl silioxane phase) coupled to a CP-WAX 52CB (25 m×0.25 mm I.D., 0.2 μm d_f, polyethylene glycol phase), both supplied by Chrompack. The CP-Sil 5CB column was connected to the injector.

The sampling conditions for the HS4O headspace were as follows: the thermostatting, transfer and needle line temperatures were maintained at 120, 150 and 150°C, respectively. Thermostatting, injection and pressurisation times were 20 min, 0.05 s and 3.0 min, respectively.

The GC conditions were as follows: the oven temperature programme was 70°C, initially held for 3 min, then raised at 6°C/min to 180°C and held for 6 min. The column head pressure was 206.8 MPa helium. A split flow of 50 ml/min was maintained. The injector and detector temperatures were 200 and 250°C, respectively. The data were processed using the PeakPro system.

The mass spectrometer used was a TRIO 1000 (Thermosperations, Staffs., UK). The scan range was 35–350 u. A scan time of 0.9 s and an interscan time of 0.1 s were used. The source and transfer line temperature were held at 200°C. For the mass spectral data only the CP-Wax 52CB column was used. The data were processed using personal computer-based VG Masslab software.

2.2.3. On-column GC

Direct on-column analysis was performed on a Fisons GC8000 gas chromatograph fitted with a cool on-column injector and a TRIO1000 mass spectrometer. The column used was a CP-Sil 5CB (25 m×0.25 mm I.D., 1.0 μm d_f). Samples of 1.5 μl were injected manually. The temperature programme was 40°C followed by a 6°C/min ramp up to 180°C and held for 5 min. The helium carrier gas head pressure was 68.9 MPa.

Mass spectrometer conditions were identical to those used with the headspace. Electron impact (EI+) ionisation was employed. The scan range was 35–350 u. A scan time of 0.9 s and an interscan time of 0.1 s were used. The transfer line and source temperature were 200°C.

2.3. Sample and standard preparations

2.3.1. HPLC

A series of diluted standards were made of sodium lactate and the degraded L-lactic acid with concentrations of 50, 100, 150 and 200 μg/ml in water. L-Lactic acid (>99%) and L-lactic acid (around 90%) standards (100 μg/ml) were made by dilution in water.

The 100 μg/ml standards of three lactic acids (degraded and 90% purity lactic acid and sodium lactate) were hydrolysed. This was achieved by adding 0.5 ml NaOH to 5 ml of sample standards. The solutions were then placed in a boiling water bath for 20 min, cooled to room temperature and neutralised with 0.5 ml orthophosphoric acid.

The two placebo formulations were prepared by extraction with water. The sample was weighed into a centrifuge tube and three extractions with water (about 15 ml) were performed. The sample was vortexed and then centrifuged at 3000 rpm after each extraction step.

2.3.2. Headspace GC

Standards of L-lactic acid (>99%) and sodium lactate were prepared by serial dilution giving 300, 400, 500, 600 and 700 μg/ml lactic acid concentrations. These solutions (20 μl) were pipetted into headspace vials and 10 μl each of methanol and saturated $NaHSO_4$ solution were added. Base placebo (5 g) was extracted by the same procedure as described for the HPLC method. Standards (1000 μg/ml) of L-lactic acid and sodium lactate were prepared in water and these were used for headspace GC–MS analysis.

2.3.3. On-column GC

A 0.03 *M* oxalic acid solution in methanol was prepared. Standards of L-lactic acid (around 90% purity), L-lactic acid (degraded) and sodium lactate (all 1000 μg/ml) were made up in the 0.03 *M* methanolic oxalic acid.
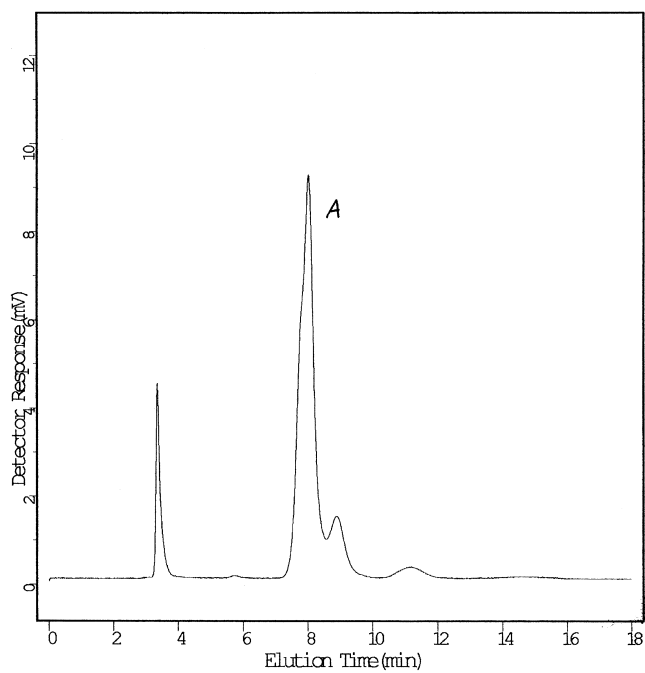
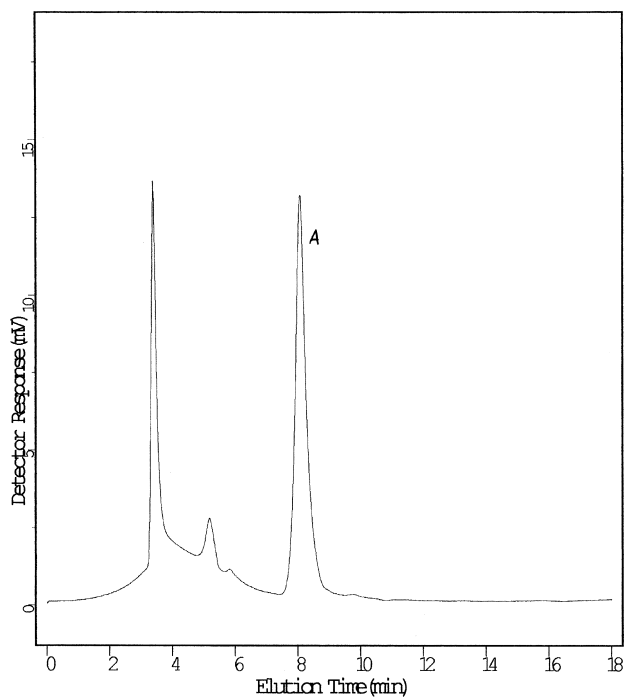Fig. 1. L-Lactic acid (A, 98% degraded), no hydrolysis.

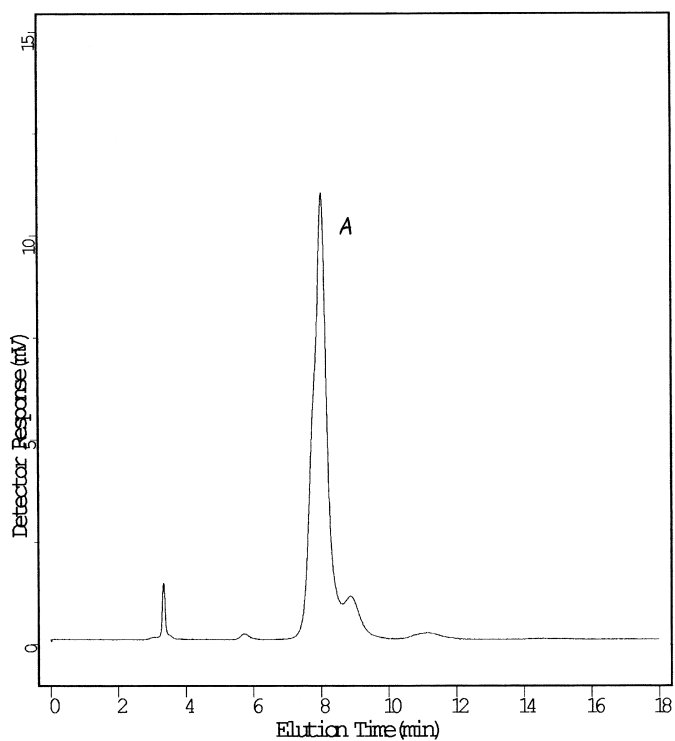Fig. 2. L-Lactic acid (A, around 90% purity), no hydrolysis.

Fig. 3. L-Lactic acid (A, >99%), lactic acid retention time 11.5 min at a flow-rate of 0.5 ml/min.
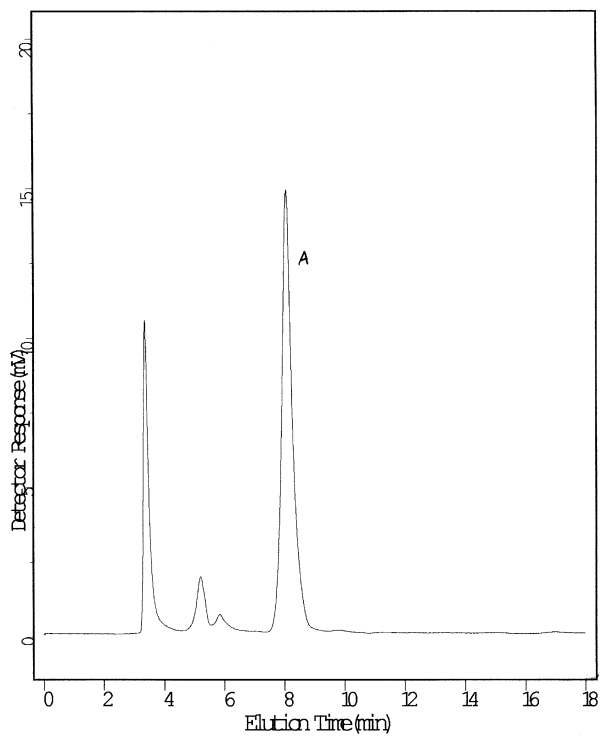

Fig. 4. Sodium lactate (A).

3. Results and discussion

3.1. HPLC

Chromatograms of L-lactic acid (degraded, Fig. 1) and L-lactic acid (around 90%, Fig. 2) show incomplete resolution of peaks. In Figs. 3 and 4 the chromatograms corresponding to L-lactic acid (>99%) and sodium lactate both give a single peak at a retention time of around 8 min.

The calibration data for the sodium lactate gives a straight line calibration with regression line $y = 5.1 + 22.48x$ with residual sum of squares 1111.9 and a correlation coefficient of 0.99999 with five data points. Five injections of a 150 μg/ml standard gave 0.1% RSD with a mean tailing factor for the lactic peak of 1.15. The degraded lactic acid calibration was obtained through summation of all the related peaks. This gave a regression line of $y = 0.6 + 1.26x$, a residual sum of squares of 0.740 and a correlation coefficient of 0.99998. The precision of four injections of a 150 μg/ml standard gave a RSD of 0.1%.

After hydrolysis the L-lactic acid (degraded, Fig. 5) and the L-lactic acid (around 90% purity, Fig. 6) showed single peaks with a retention time of 8 min.

No interfering peaks were observed with the placebo formulations. However, after hydrolysis interfering peaks were observed for placebo formulation A (Fig. 7) and placebo formulation B (Fig. 8).

3.2. Headspace GC

A typical chromatogram of L-Lactic acid by headspace GC is shown in Fig. 9. The response was linear over the range 300–700 μg/ml with correlation coefficients between 0.990 and 0.999. The RSD for five injections of the 500 μg/ml standard for both L-lactic acid (>99% purity) and sodium lactate gave values of typically 4–5%.

A base formulation sample chromatogram is shown in Fig. 10. The peak purity was confirmed using mass spectrometry. A total ion chromatogram (TIC) of L-lactic acid (>99%) is shown in Fig. 11 and was confirmed by mass spectrometry.
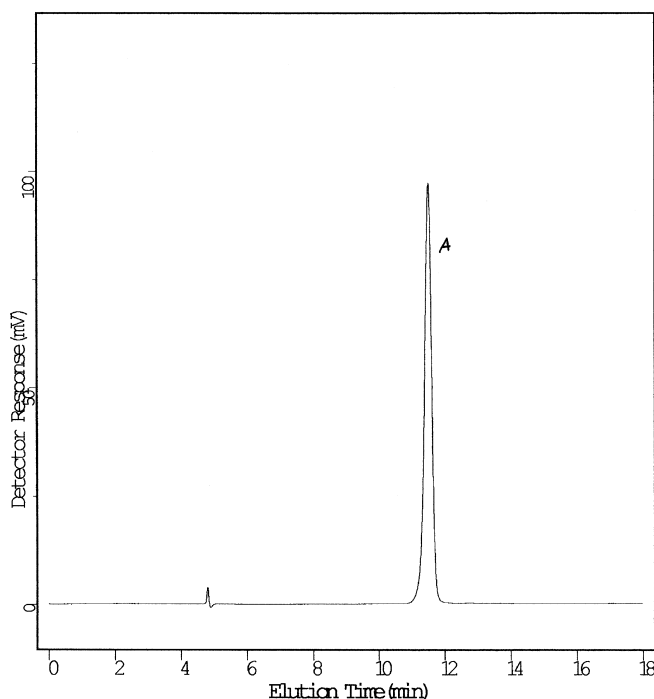
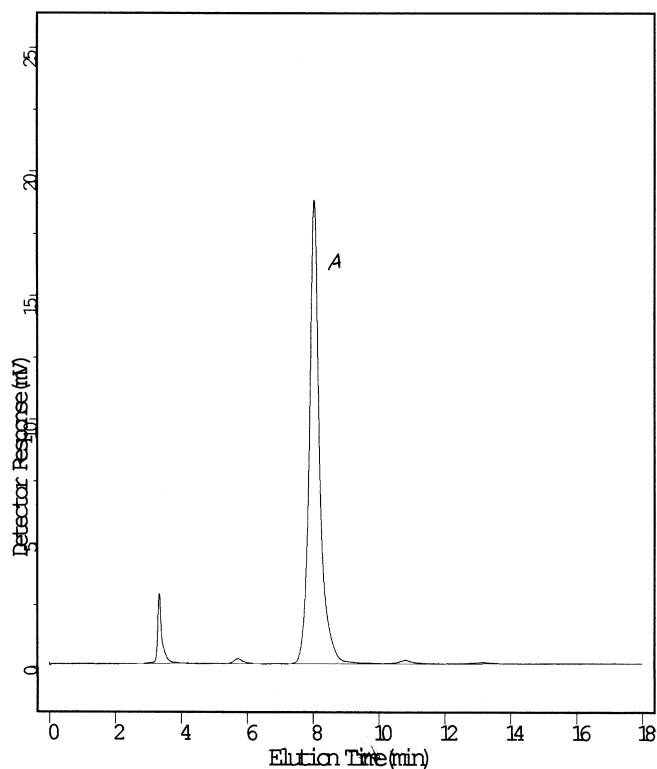Fig. 5. L-Lactic acid (A, 98% degraded), hydrolysed.

Fig. 6. L-Lactic acid (A, around 90% purity), hydrolysed.
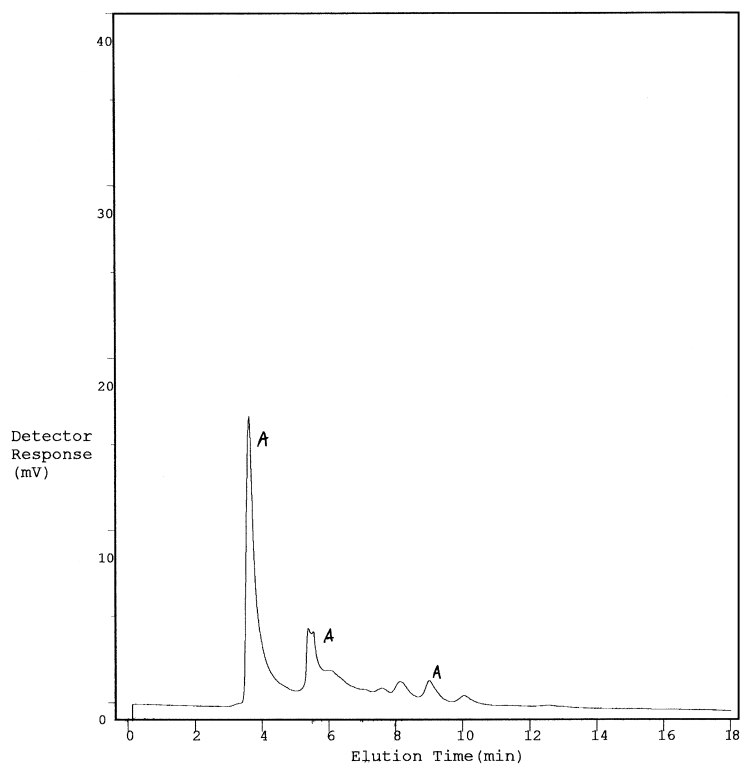

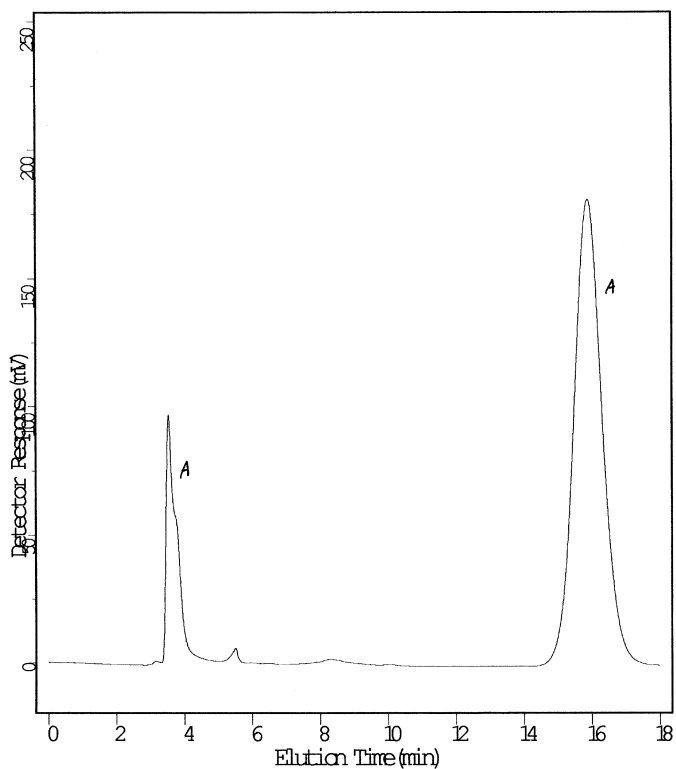Fig. 7. Placebo formulation A after hydrolysis (A: excipient peaks).

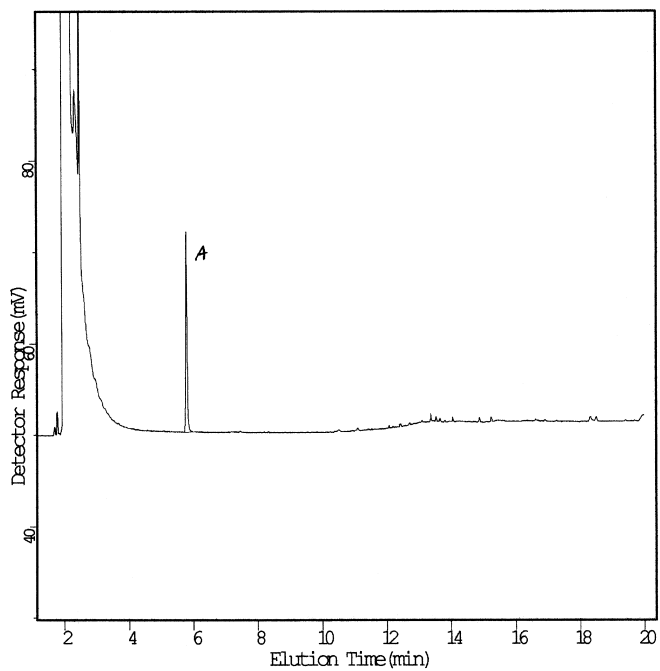Fig. 8. Placebo formulation B after hydrolysis (A: excipient peaks).

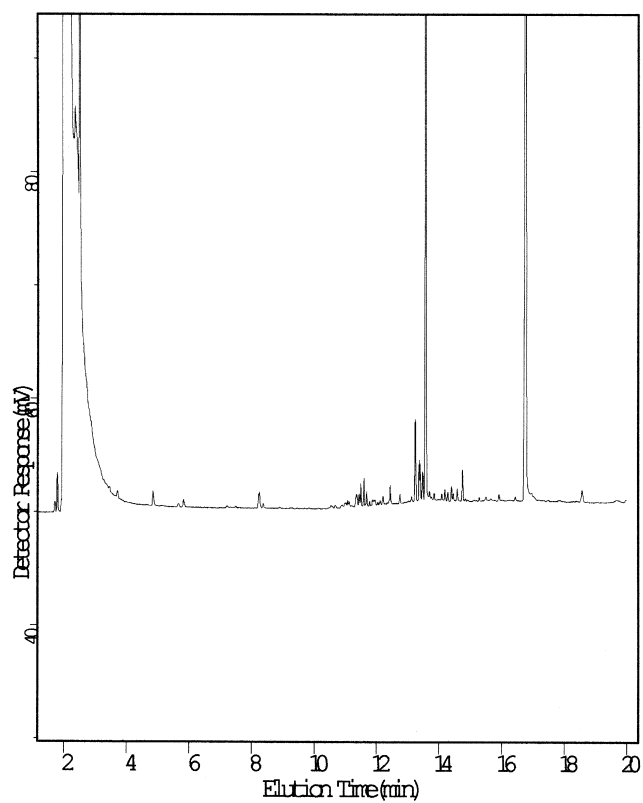Fig. 9. L-Lactic acid (99% purity). Peak A is lactic acid.
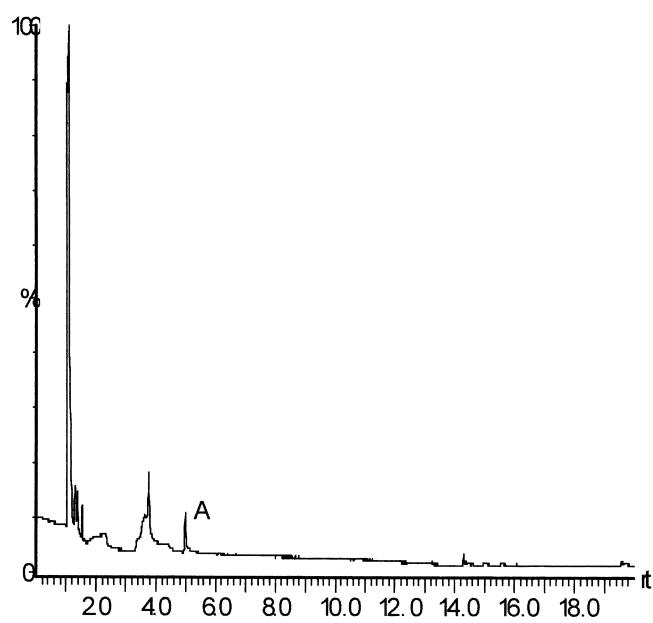
Fig. 10. Base formulation (no lactic acid).

Fig. 11. TIC of L-lactic acid (Fluka). Peak A is lactic acid; rt, retention time (min).

3.3. On-column GC–MS

The sodium lactate total ion current (Fig. 12) gave a single peak not attributable to the solvent with a retention time of approximately 4.5 min.

The degraded L-lactic (Fig. 13) and the 90% purity lactic acid (Fig. 14) gave peaks at 4.5, 13.7 and 22.2 min.

A plot of the retention times of the three peaks gives a linear relationship with a residual sum of squares of 0.0777 and a correlation coefficient of 0.99975.

The mass spectra of the peaks at 4.5, 13.7 and 22.2 min are shown in Figs. 15–17, respectively.

There are various modes of action of lactic acid/ lactate in consumer healthcare products and this determines the analytical requirements of the method, such as, for example, the use of sodium lactate or lactic acid in formulations. The grade of lactic acid used is important, as the polylactides that are present in many supplies may need to be separated from lactic acid and quantified.

Lactic acid can be obtained in various grades to greater than 99%. However, for this study the 98% purity batch was degraded. The lower grade of lactic acid (about 90%) is normally used in consumer healthcare formulations. It is clear from the results (Figs. 1–6 and Figs. 13–17) that both the lower grade lactic acid and the degraded L-lactic acid gave rise to the polylactide peaks. It can be concluded that given time and the right conditions all L-lactic acid batches will produce polylactides.

The HPLC method detailed in this paper shows linearity and a 0.1% RSD when using sodium lactate. Linearity remains consistent and the precision comparable when lactic acid was used for calibration (without being hydrolysed) with polylactide interfering peaks summed into the calibration. As the summation of the polylactide peaks is not an acceptable means of quantifying total lactic acid in the formulation, the method was developed with a sample hydrolysis stage. The hydrolysis step converts the polylactides into lactic acid thus giving gaussian peaks (see chromatograms in Figs. 1–6).
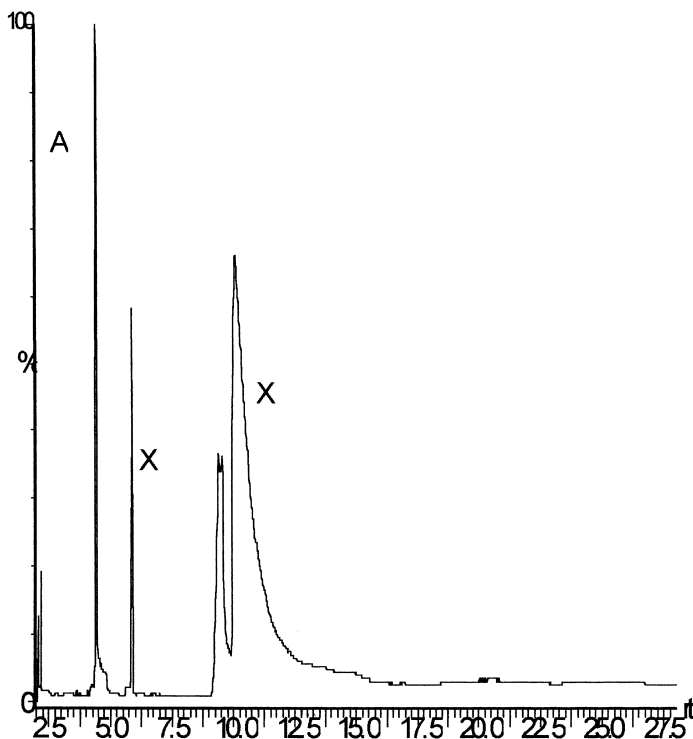

Fig. 12. Sodium lactate. A, lactic/lactate peak; X, solvent excipients; rt, retention time (min).
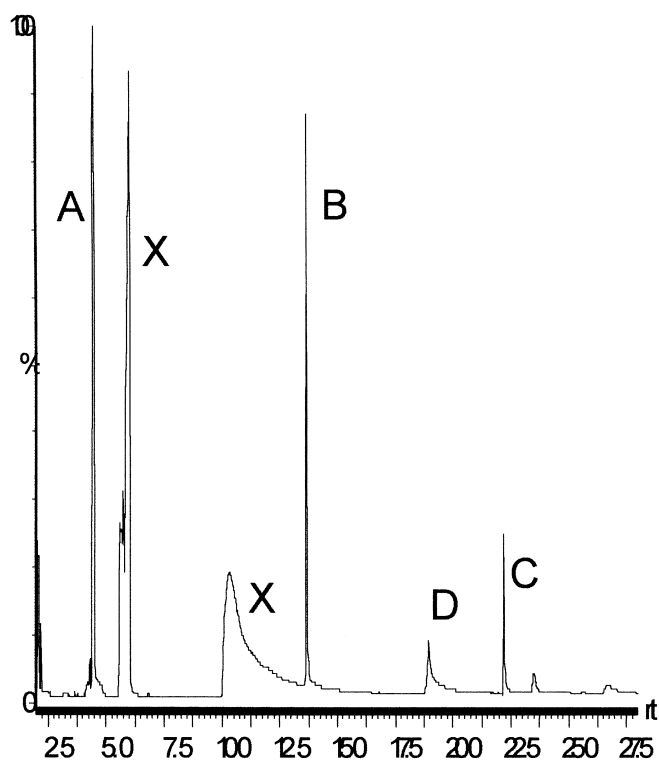
Fig. 13. L-Lactic acid (degraded). A, Lactic acid; B, lactic acid dimer (lactoyl lactic acid); C, lactic acid trimer; D, unknown; X, solvent excipients; rt, retention time (min).

However, sample hydrolysis has the disadvantage of being time consuming and can often lead to hydrolysis of matrix excipients that give interfering peaks (see Figs. 7 and 8).

The use of a dual column system for the headspace GC method gave greater selectivity to the method with resultant separation of peaks of interest from the matrix constituents. Lactic acid, sodium lactate and the polylactides were all converted to methyl lactate; the conversion produces a single methyl lactate peak (Fig. 11), as confirmed by the mass spectrometer data, and is observed with a retention time of 5.8 min. The method yields a linear response between 300 and 700 µl/ml and a RSD of 4–5% RSD.

The precision described consists of a combination of instrumental and method precision. The RSD values of 4–5%, whilst being acceptable, may be improved by using an internal standard.

On-column GC was used to quantify lactic acid and polylactides. The rationale behind the use of on-column GC was to effect a gentler GC sample introduction, thus removing the possibility of in-situ degradation of components. Peaks were identified using mass spectra for the low purity and degraded L-lactic acid total ion chromatograms. The linear relationship of the plot of the lactic and polylactides peak retention times indicated that they are a homologous series. This was confirmed by their mass spectral data. The peak with a retention time of 4.5 min was lactic acid (molecular mass m/z 90), as deduced by mass spectral interpretation, and the peak with a retention time of 13.7 min had a molecular mass (m/z) of 162; i.e. corresponding to lactoyl lactic acid (dimer), and this was deduced as the condensation of two molecules of lactic acid. The molecular ion peak with a retention time of 22.2 min was not visible. This compound had a similar fragmentation pattern (as shown in Figs. 15–17), but with some higher mass fragments than the other two peaks. The sodium lactate TIC gave a peak with a retention time of about 4.5 min.
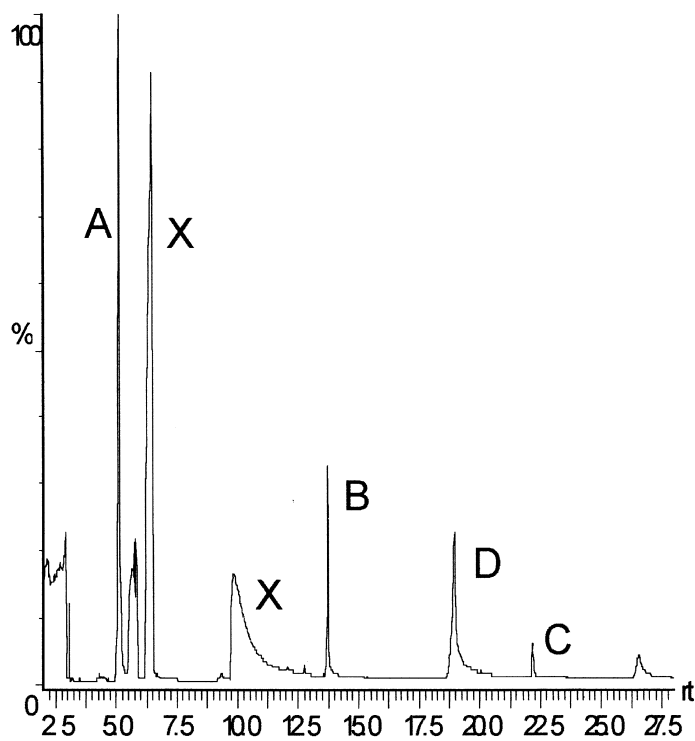
Fig. 14. L-Lactic acid (around 90% purity). A, Lactic acid; B, lactic acid dimer (lactoyl lactic acid); C, lactic acid trimer; D, unknown; X, solvent excipients; rt, retention time (min).
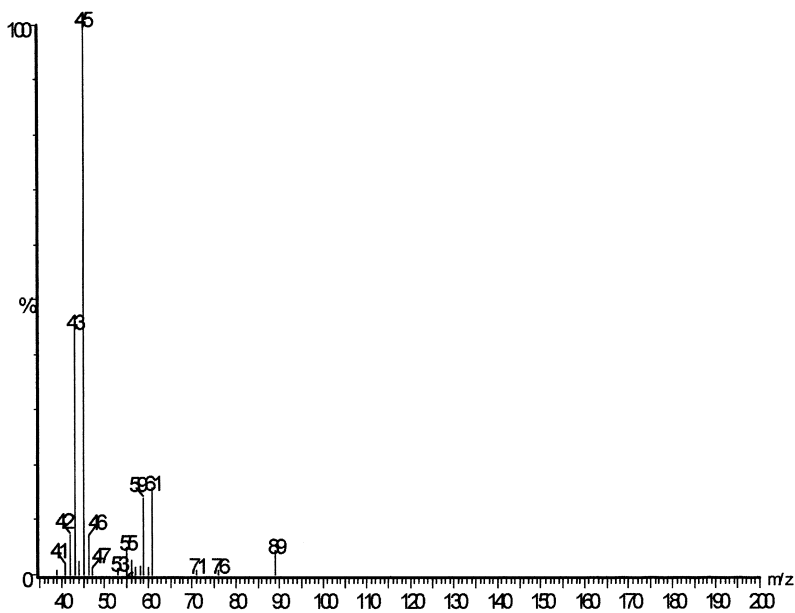

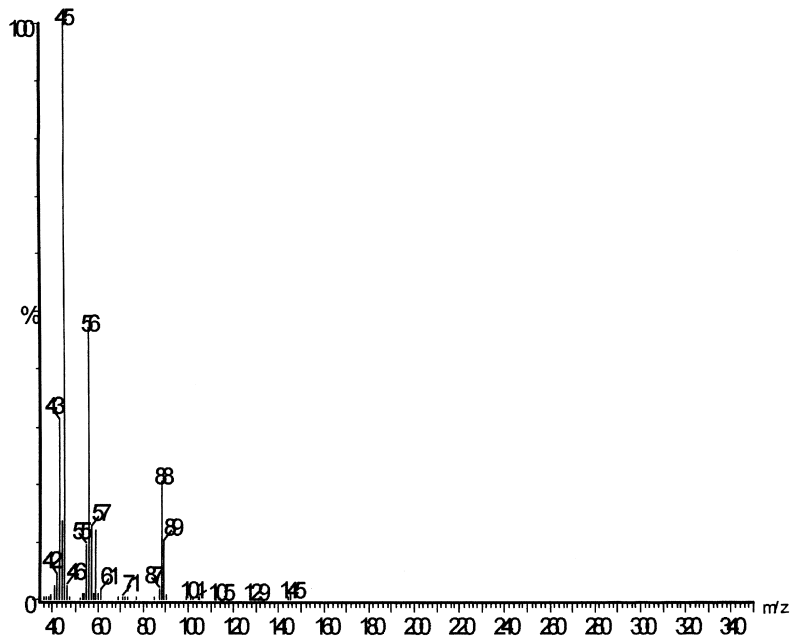Fig. 15. Mass spectrum of peak A in Figs. 13–15.

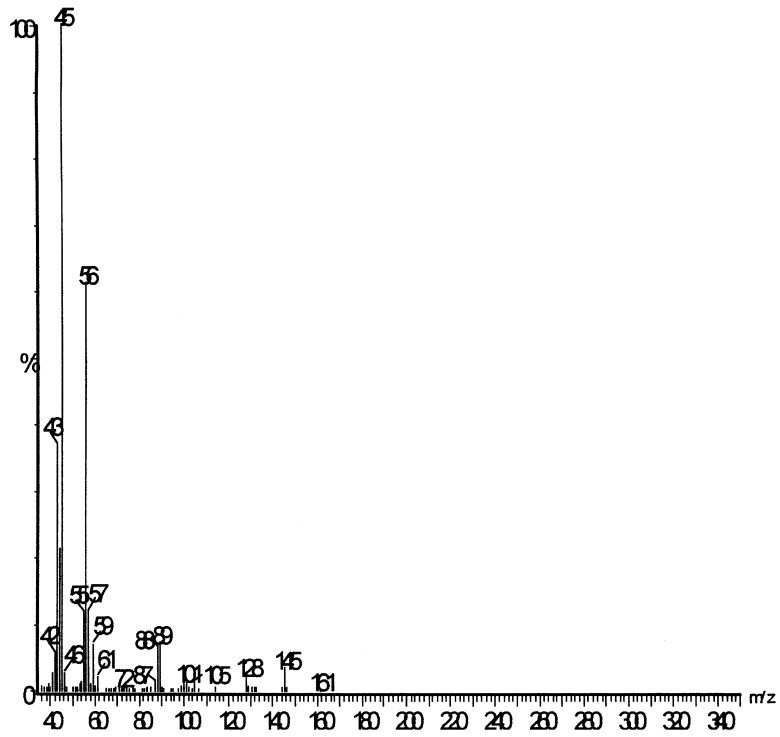Fig. 16. Mass spectrum of peak B in Figs. 14 and 15.

Fig. 17. Mass spectrum of peak C in Figs. 14 and 15.

From Figs. 13 and 14, peak A is still confirmed as lactic acid from the mass spectral data, but there is a change in retention time. The only explanation that can be offered for this is that the standard used the 90% grade and this contains up to 10% water and the effect of the water on the stationary phase has altered the retention characteristics.

The formulation of a consumer healthcare product with sodium lactate is recommended over the use of lactic acid for two reasons. Firstly, the quality of sodium lactate analysis by the HPLC method detailed here is easier than the corresponding lactic acid analysis due to chromatographic considerations of the polylactides. Secondly, using lactic acid will introduce polylactide 'impurities' (as shown by on-column GC–MS; Figs. 12–17) into the formulation and thus potentially affect the efficacy of the product.

Due to the presence of polylactides in lactic acid the choice of analytical method used must reflect this. For instance, if the activity of the therapeutic product is affected by the polylactides, then polylactides need to be quantified and therefore the method of choice is on-column GC. For formulations where lactic acid is used (this being liable to degradation/ polymerisation) then either hydrolysis followed by HPLC or headspace GC is recommended as the means of analysis

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