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# Optimisation and validation of a rapid and efficient microemulsion liquid chromatographic (MELC) method for the determination of paracetamol (acetaminophen) content in a suppository formulation

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**Abstract**

A rapid and efficient oil-in-water microemulsion liquid chromatographic method has been optimised and validated for the analysis of paracetamol in a suppository formulation. Excellent linearity, accuracy, precision and assay results were obtained. Lengthy sample pre-treatment/extraction procedures were eliminated due to the solubilising power of the microemulsion and rapid analysis times were achieved. The method was optimised to achieve rapid analysis time and relatively high peak efficiencies. A standard microemulsion composition of 33 g SDS, 66 g butan-1-ol, 8 g *n*-octane in 1 l of 0.05% TFA modified with acetonitrile has been shown to be suitable for the rapid analysis of paracetamol in highly hydrophobic preparations under isocratic conditions. Validated assay results and overall analysis time of the optimised method was compared to British Pharmacopoeia reference methods. Sample preparation and analysis times for the MELC analysis of paracetamol in a suppository were extremely rapid compared to the reference method and similar assay results were achieved. A gradient MELC method using the same microemulsion has been optimised for the resolution of paracetamol and five of its related substances in approximately 7 min.
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*Keywords:* Microemulsion liquid chromatography; Paracetamol; Acetaminophen; Suppository; Validation; Related substances; Gradient elution

## 1. Introduction

Microemulsion liquid chromatography (MELC) is a relatively new chromatographic technique, which utilises microemulsions as a mobile phase and has been shown to be suitable for the separation of a range of pharmaceutical compounds using both isocratic and gradient elution modes [1,2] and for validated determinations of fosinoprilat in human plasma [3] and simvastatin and its impurities in bulk drug and tablet formulations [4]. Oil-in-water microemulsions are composed of nanometre sized droplets of a water immiscible liquid (oil) dispersed throughout an aqueous continuous phase, these oil droplets are stabilised by the presence of a surfactant and a co-surfactant which reduce the interfacial tension at the oil/water interface to almost zero, resulting in a stable system. Microemulsions possess a unique property in that they can solubilise both polar and non-polar substances due to the arrangement of the oil and aqueous phases. Due to the high aqueous content of O/W microemulsions, they are very compatible with reversed-phase HPLC columns while the hydrophobic oil core gives them the ability to dissolve non-polar solutes and sample matrices.

Paracetamol, also known as acetaminophen is an analgesic and antipyretic which is popular throughout the world and is used as a treatment for pain relief and fever [5] and is considered safe for use in a variety of patients including the elderly, pregnant women and children. Paracetamol is available as a non-prescription drug in a variety of over the counter preparations such as soluble, insoluble and dispersible tablets, oral suspensions and solutions, and suppositories [6].

Generally when water-soluble pharmaceutical compounds are present in non-polar matrices such as creams, ointments or suppositories, dissolution of the non-polar matrix in a suitable

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solvent and extraction of the active compound is required before analysis is possible. Various methods such as calorimetry [7], spectrophotometry [8] and HPLC with inverse supercritical extraction [9] have been reported for the assay of paracetamol in suppositories. The latter method reported an improvement in analysis times compared to US Pharmacopoeia (1998) [10] method but still required a number of time consuming extraction steps before HPLC analysis was possible. The British Pharmacopoeia 2005 [11] details a titrimetric assay method, which requires refluxing five individual suppository samples in acid for 1 h, followed by a number of intermediate steps before titration and calculation of a mean assay value.

In this study, a commercially available paracetamol suppository preparation, Paralink™, was obtained and a previously reported MELC method which was first used by Marsh et al. [1,2] for the analysis of a number of pharmaceutical compounds was optimised for the rapid determination of paracetamol content. This method used a microemulsion composition of 33 g SDS, 66 g butan-1-ol, 8 g *n*-octane in 1 l of 0.05% TFA.

In this work the method was optimised in terms of application to paracetamol analysis. Column temperature and the addition of organic solvents to the microemulsion at various concentrations were investigated and found to affect peak efficiency and retention times. Peak asymmetry was also evaluated but was not effected by either temperature or organic additives. Sample preparation was also relatively rapid and required sonication in the microemulsion, filtration and direct injection onto the column which removed the necessity for extraction procedures, thereby dramatically reducing analysis times.

Validation of the analysis of paracetamol in a suppository was carried out according to ICH guidelines [12] for linearity, accuracy, precision, LOD and LOQ, using propyl paraben as an internal standard. The BP 2005 assay method for paracetamol in a suppository [11] was carried out to compare the efficiency of the method in terms of analysis times, sample preparation, assay results and general ease of use.

An important aspect of pharmaceutical analysis is the detection and quantitation of related substances in formulated products and bulk drug substances. These related substances may be degradation products or precursors to the synthesis of the active ingredient. Marsh et al. [2] used MELC with gradient elution to separate the degradants and active ingredients present in a number of pharmaceutical formulations. In order to assess the stability indicating capability of MELC for paracetamol related substances, a sample of paracetamol was spiked with five known related compounds which could potentially be present in formulated products or bulk drug substances. Isocratic and gradient MELC methods were assessed for resolution of paracetamol from five of its related impurities. A gradient MELC method was successfully developed for the rapid resolution of paracetamol and five related substances plus one unknown compound. A previous study by Nageswara [13] utilised reversed-phase gradient HPLC for the separation of paracetamol and nine related substances where reversing the polarity of the mobile phase during the separation achieved resolution of all substances. As oil-in-water microemulsions are composed mainly of water, performing gradient elution by ramping up the concentration of the aqueous component does not reverse the polarity of the eluent and co-eluting compounds can be separated by exploiting differences in their hydrophobicity.

It is concluded that MELC offers a rapid and efficient method for the analysis of paracetamol in suppository formulations. We expect that MELC will be increasingly applied to the analysis of pharmaceuticals, in particular where the solubilising power and versatility of the microemulsion diluent offers efficiency gains for sample preparation.

## 2. Experimental

### 2.1. Chemicals

Propyl paraben, paracetamol and its related substances; 4-nitrophenol (precursor), 4-aminophenol (degradation product /precursor), 4-chloroacetanilide (side product), 4-hydroxyacetophenone (side product) and 2-acetamidophenol (side product) were obtained from Sigma–Aldrich (Ireland). The microemulsion components: HPLC grade water, *n*-octane, trifluoroacetic acid (TFA), butan-1-ol (all Romil) and 99% SDS (BDH) were obtained from Lennox Laboratory Supplies (Ireland). Organic additives: propan-1-ol and acetonitrile (Romil) were also obtained from Lennox.

Paralink suppositories containing 500 mg paracetamol were purchased over the counter from a local pharmacy. Reference assay method requirements; 1 M sulphuric acid, ferroin solution, dilute hydrochloric acid, orthophosphoric acid, and 0.2 M ammonium cerium(IV) sulphate were obtained from the chemical stores at WIT.

### 2.2. Equipment

A Hewlett Packard 1050 HPLC system equipped with HP solvent degassing module (model G1303A), HP variable wavelength UV/Vis detector (79853C), HP solvent cabinet and column heater (79856A), HP 21 station autosampler (79855A), and HP quaternary pump (79852A) coupled to an Agilent Chemstation data management system (Rev.A.09.01 [1206]) was used for all work carried out.

Method optimisation for the detection of paracetamol and for the separation of paracetamol and its related substances was carried out using a Waters Symmetryshield RP18 150 mm × 4.6 mm column with 3.5 μm packing material. To further reduce the analysis times for the suppository samples, the optimised method was transferred to a Waters Symmetry C18 100 mm × 4.6 mm column with 3.5 μm packing material.

### 2.3. Method optimisation

#### 2.3.1. Microemulsion preparation

The microemulsion reported by Marsh et al. [1], referred to as the 'standard' microemulsion was prepared by mixing 66 g of butan-1-ol, 8 g of *n*-octane and 33 g of SDS. This was sonicated for approximately 10 min until a homogeneous solution was achieved. One litre of 0.05% (v/v) TFA was then added, sonicated for 30 min and filtered. Amounts of propan-1-ol and

acetonitrile were added to the prepared microemulsion to give a range of concentrations from 0 to 30.5% (v/v) propan-1-ol and from 0 to 7.4% (v/v) acetonitrile. Above these concentrations the microemulsion broke down resulting in a cloudy dispersion of the components suggesting the formation of an emulsion. Addition of each solvent was carried out slowly while sonicating to prevent the microemulsion becoming disrupted. Above 3.8% (v/v) acetonitrile the microemulsion became very cloudy and prolonged sonication was required to re-form the microemulsion.

*2.3.2. Reference standard solution preparation*

3.9 mg of paracetamol reference standard was dissolved in approximately 15 ml of the standard microemulsion by sonicating for 5 min. The sample solution was allowed to cool and made up to 20 ml in a volumetric flask with the microemulsion.

*2.3.3. Chromatographic conditions*

The 150 mm column was used for method optimisation. The variable wavelength detector was set at 220 nm for all runs. Injection volume was 5 μl. The initial runs for all concentrations of organic modifiers were carried out with the column temperature set to 60 °C to monitor retention times and peak efficiency. The flow rate was varied between 1 and 2 ml min$^{-1}$ to monitor the retention times of both solutes and the back-pressure generated by the relatively viscous microemulsion.

*2.3.4. Optimising the microemulsion composition*

The reference sample solution was run using both the propan-1-ol and acetonitrile modified microemulsions at a range of concentrations stated in Section 2.3.1. Once the optimum microemulsion composition was determined in terms of retention time and peak efficiency, the reference standard was run using the chosen microemulsion at a range of column temperatures to monitor the effect of temperature on retention times, peak efficiency and column back-pressure.

The addition of propan-1-ol up to 5% (v/v) decreased the retention of paracetamol slightly but further increases in concentration up to 30.5% (v/v) had no effect on retention. The addition of acetonitrile up to 7.4% (v/v) resulted in a slight decrease in retention of paracetamol over the concentration range used. The peak efficiency values were calculated for paracetamol using both modified microemulsions over the same concentration ranges at a flow rate of 1.5 ml min$^{-1}$. The addition of propan-1-ol up to 5% (v/v) resulted in a decrease in peak efficiency for paracetamol from approximately 6500 to 3900 theoretical plates. Further increases in propan-1-ol concentration did not have a significant affect on peak efficiency as illustrated in Fig. 1. The addition of acetonitrile however had the opposite effect with an increase in theoretical plate numbers to approximately 11,000 with 2.5% (v/v) acetonitrile, with no significant change at higher concentrations (see Fig. 2).

The unmodified standard microemulsion had a pH of 2.8. This value was not affected to any significant degree by the addition of the organic modifiers. Paracetamol with a p$K_a$ of 9.5 [15] is completely unionised at this pH and retains its hydrophobicity. This increases its retention on the column, which increases

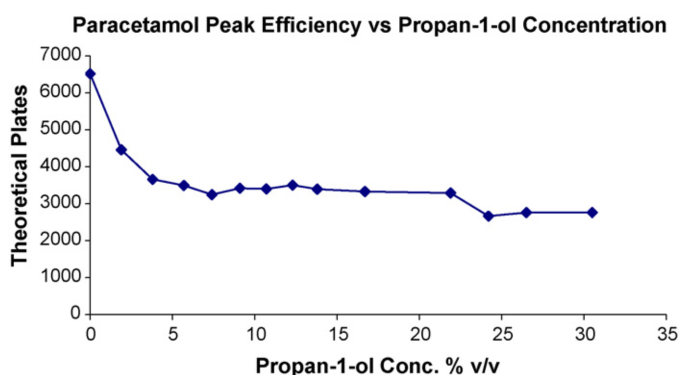


Fig. 1. A plot of paracetamol peak efficiency vs. % (v/v) propan-1-ol showing the effect of propan-1-ol addition on peak efficiency.

retention times [1]. As the retention time of paracetamol was already very short, optimising the pH was not carried out.

Asymmetry values for the paracetamol peaks were not affected by the addition of organic modifiers or temperature changes and a peak asymmetry value ($A_s$) of ∼1.7 was constant for all runs. Although validated analytical techniques require $A_s$ to be less than 1.2, peak asymmetry was not optimised and will be addressed in future work.

*2.3.5. Optimising column temperature and flow rate*

The optimum microemulsion composition for the analysis of paracetamol was chosen to be the standard microemulsion plus 3% (v/v) acetonitrile as this was the easiest microemulsion to prepare while still providing high efficiency values and low retention times. The propan-1-ol modified microemulsion was used to monitor the effects of column temperature on column back-pressure, retention times and peak efficiencies. The temperature range used was 25–60 °C. It was found that increasing the column temperature over the chosen range reduced the retention time of paracetamol only fractionally. Similarly, peak efficiency values were not affected to any significant degree and a slight increase was noted. There was however a significant drop in column back-pressure as the temperature was increased. The

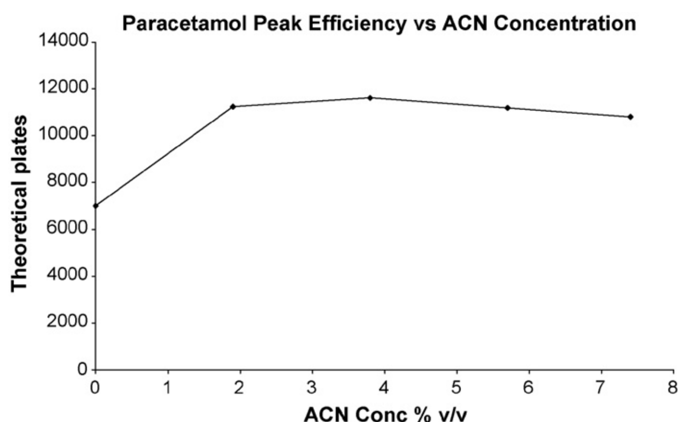


Fig. 2. A plot of paracetamol peak efficiency vs. % (v/v) acetonitrile showing the effect of acetonitrile on peak efficiency.

viscosity of the microemulsion was measured over the same temperature range and it was found that column temperature, column back-pressure and microemulsion viscosity showed a direct relationship. When choosing the column temperature and flow rate it was decided that a column temperature of 50 °C and a flow rate of 1.75 ml min$^{-1}$ were the optimum values to give short retention times and keep column back-pressure well below the instruments limit.

### 2.3.6. *Transfer of the method to a shorter column*

As is common practice in HPLC method development, an optimised method can be transferred to a shorter column to further reduce analysis times. In this case the method was successfully transferred to a Waters Symmetry C18 100 mm × 4.6 mm column with 3.5 μm packing material resulting in a further reduction in analysis time.

### 2.3.7. *Choosing an internal standard*

It was known from previous experiments [2] that a range of paraben preservatives eluted quickly in the order of methyl, ethyl and propyl paraben when using the standard microemulsion as an eluent in MELC. After spiking the paracetamol solution separately with methyl, ethyl and propyl parabens, propyl paraben was chosen as this and was completely resolved from paracetamol, eluted rapidly and was not present in the suppository formulation.

## 3. Sample preparation

Each suppository contained 500 mg of paracetamol. To determine the optimum concentration of suppository that could be solubilised, i.e. the highest concentration in the shortest time, a range of concentrations of the suppository in the standard microemulsion were sonicated. The chosen concentration was 10 mg of the suppository solubilised in 100 ml of the standard microemulsion in 15–20 min. This had a theoretical concentration of 0.02 mg ml$^{-1}$ paracetamol. Sample preparation consisted of sonication for between 15 and 20 min followed by syringe filtration with a total preparation time of no more than 20–25 min.

## 4. Validation

### 4.1. *Specificity*

Paralink suppositories contain 500 mg paracetamol with hard fat and polyoxyl 40 stearate as inactive excipients. Paracetamol is the only UV active component in the preparation, therefore, the method is specific for the analysis of paracetamol.

### 4.2. *Linearity*

A stock solution of paracetamol reference standard was prepared quantitatively by dissolving 100 mg of paracetamol reference standard in 100 ml of the standard microemulsion. A 1 in 10 dilution was performed on this solution to give a working standard solution of 0.1 mg ml$^{-1}$. A 0.5 mg ml$^{-1}$ solution of propyl paraben in the standard microemulsion was prepared as the internal standard. A range of five standard solutions of paracetamol reference standard was prepared from stock in the range 0.004–0.02 mg ml$^{-1}$. Each standard contained 0.06 mg ml internal standard.

Ten microlitres of each standard solution was injected and run in triplicate using the optimised chromatographic conditions. Mean peak areas for paracetamol and the internal standard were calculated. A calibration curve was plotted of peak area ratios of paracetamol/internal standard (P/I) against concentration.

### 4.3. *Assay*

As a comparison to the reference assay method, five suppositories were accurately weighed and a mean weight was calculated. Each suppository was to have contained 500 mg paracetamol (label claim). It was determined that each suppository contained 22% (w/w) paracetamol. The five suppositories were crushed and a 0.1 mg ml$^{-1}$ solution of the suppository was prepared as described earlier. Twelve millilitres of this solution was added to 3 ml of the internal standard solution and made up to 25 ml with the standard microemulsion. Ten microlitres of this solution was injected and run in triplicate. A sample chromatogram is shown in Fig. 3. Mean peak areas for paracetamol and the internal standard were calculated and the concentration of paracetamol in the sample was calculated using P/I and the calibration curve equation. Assay values were compared to label claim as percent paracetamol recovered.

### 4.4. *Precision—repeatability*

Three sets of three replicate concentrations of the suppository were prepared. Three separate sample stock solutions (0.1 mg ml$^{-1}$) were first made up. Eight, 12 and 15 ml of each sample solution was added to 3 ml of the internal standard solution and made up to 25 ml with the microemulsion. Each of the nine sample solutions was run in triplicate. Repeatability was determined by calculating the relative standard deviation (R.S.D.) of the determined paracetamol concentrations.

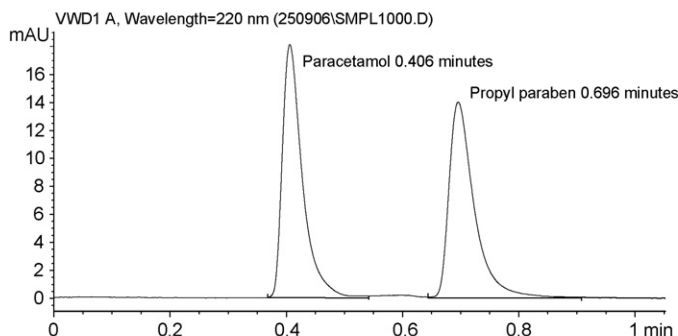


Fig. 3. Sample chromatogram of Paralink suppository solution spiked with internal standard. One hundred milligrams of Paralink suppository in 100 ml standard microemulsion (sample sock solution). Twelve millilitres of sample stock + 3 ml of propyl paraben as internal standard made up to 25 ml. Ten microlitres injection, 1.75 ml min$^{-1}$ isocratic elution, standard microemulsion + 3% (v/v) acetonitrile, 100 mm × 4.6 mm × 3.5 μm C18 column (50 °C, 220 nm).

### 4.5. Intermediate precision

Each of the three sample stock solutions described were run on different days under identical conditions. Intermediate precision was determined by the R.S.D. of the calculated concentrations.

### 4.6. Accuracy

To determine the accuracy of the method, a range of eight known concentrations of the paracetamol reference standards (0.017–0.001 mg ml$^{-1}$) were run in triplicate and the concentration for each was determined from the calibration curve. Results were expressed as percent recovery of the known concentration.

### 4.7. Limit of detection (LOD)

The LOD was calculated based on the calibration curve using the following equation:

$$\mathrm{LOD} = \frac{3.3\sigma}{S} \quad (1)$$

where $\sigma$ is the residual standard deviation of the regression line and $S$ is the slope. $\sigma$ was calculated using the following equation:

$$\sigma = \frac{\sqrt{\sum (y_i - \hat{y}_i)^2}}{n-2} \quad (2)$$

### 4.8. Limit of quantitation (LOQ)

The LOQ was calculated using the following equation:

$$\mathrm{LOQ} = \frac{10\sigma}{S} \quad (3)$$

## 5. Reference method

The assay for paracetamol in the suppositories was carried out based on the method described in the British Pharmacopoeia 2005 [11]. The method required obtaining a mean assay value from five separate suppository samples. This lengthy procedure required individually refluxing five suppositories in an acid solution followed by a number of sample treatment procedures and titration with ammonium cerium(IV) sulphate. The method was followed as stated except that three suppositories were analysed.

## 6. Stability indicating study

To assess the stability indicating capability of MELC for paracetamol, five related substances, which are either precursors to paracetamol synthesis, side products or its degradation products were obtained. The BP 2005 test for paracetamol related substances [14] describes a HPLC method for the determination of 4-aminophenol, 4-nitrophenol and 4-chloroacetanilide. 4-Hydroxyacetophenone and 2-acetamidophenol are side products [13] potentially present in paracetamol bulk substance and/or formulations.

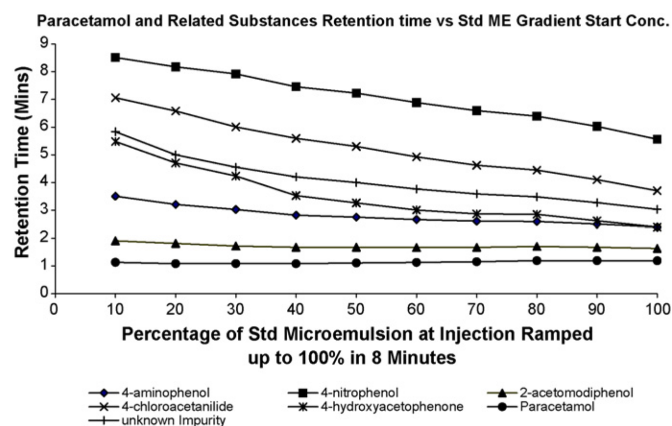


Fig. 4. Variation in retention times of paracetamol and related substances with microemulsion gradient.

A solution of 2.6 mg paracetamol reference standard in the standard microemulsion was spiked with 0.2 mg of each of the related compounds listed Section 2.1 and made up to 25 ml. Ten microlitres of this solution was injected and run on the 150 mm column at 40 °C using the standard microemulsion. The detection wavelength was 220 nm. A number of flow rates were selected in an attempt to obtain resolution of all six compounds. An isocratic flow rate of 1.2 ml min$^{-1}$ yielded the best resolution however, 4-aminophenol and 4-hydroxyacetophenone co-eluted.

In order to resolve all peaks, a number of gradient modes were attempted. Fig. 4 illustrates the change in retention times of paracetamol and all five related compounds plus one unknown impurity. The initial gradient conditions were (90/10, microemulsion/0.05% TFA) ramped up to 100% microemulsion in 8 min. The proportion of microemulsion in the mobile phase at injection was progressively reduced and the retention times of all compounds were plotted against gradient conditions at injection. All compounds were successfully resolved along with one unknown impurity present in one of the reference standards. The optimum gradient conditions chosen to resolve all compounds in the shortest time was (50/50, microemulsion/0.05% TFA), ramped up to 100% microemulsion in 8 min, see Fig. 5. The method was optimised for the separation of paracetamol and related substances to demonstrate the ability of gradient MELC to be used for rapid stability indicating studies. Further quantitative analysis and validation using internal standards will be the subject of future work.

## 7. Results and discussion

The objectives of this study were the optimisation and validation of a rapid MELC method for the determination of the active pharmaceutical ingredients in formulations composed of highly hydrophobic matrices, while eliminating sample pre-treatment and extraction procedures. The target of this study, a paracetamol suppository was chosen because suppositories generally require a number of sample extraction and/or pre-treatment steps before the analysis can be performed [7–9,11] due to the hydrophobic bulk ingredient in the preparation. Sample preparation was

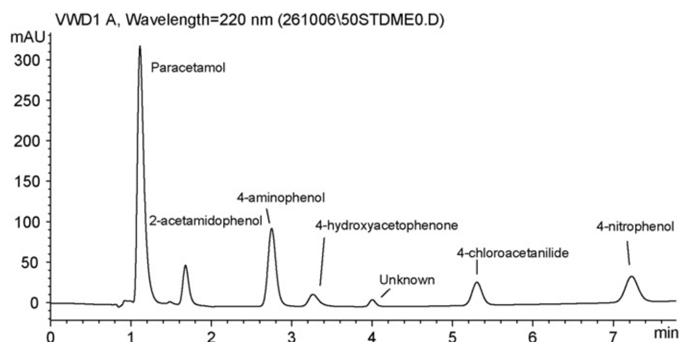


Fig. 5. Separation of paracetamol and related substances using gradient elution. Flow rate 1.2 ml min$^{-1}$, 150 mm × 4.6 mm × 3.5 μm C18 column, 40 °C. Gradient conditions (50/50, microemulsion/0.05% TFA) ramped up to 100% microemulsion over 8 min.

shown to be extremely rapid compared to the reference method and required a single step of dissolving the suppository sample in the microemulsion. The standard microemulsion was chosen as the sample diluent as this displayed a higher solubilising power than when modified with acetonitrile. In addition to the dramatic reduction in sample preparation time, a minimal amount of equipment was required compared to the BP reference method, i.e. five suppositories requires five sets of reflux apparatus for simultaneous sample preparation.

The standard microemulsion composition which had previously been used by Marsh et al. [1,2] was optimised for the analysis of paracetamol with respect to retention times and peak efficiency. Paracetamol was hardly retained on the column and the modest reduction in its retention time due to the addition of organic modifiers was expected. While the addition of acetonitrile increased the efficiency of paracetamol peaks, propan-1-ol had an opposite affect and reduced peak efficiency. It was found that microemulsion viscosity and column back-pressure were directly dependent on the column temperature and a flow rate of 1.75 ml min$^{-1}$ with a column temperature of 50 °C was the optimum to keep back-pressure and retention times low. A microemulsion composition of 3% (v/v) ACN in the standard microemulsion was the optimum for paracetamol and analysis.

Validation of the paracetamol method showed excellent results for linearity ($r^2 = 0.9999$), accuracy (R.S.D. = 99.75%) and precision (repeatability: R.S.D. = 0.7%, int. prec. R.S.D. = 0.84%). While an assay value of 100.1% compares very favourably with the reference assay of 99.8%, see Table 1. These results indicate that the optimised MELC method is suitable for routine rapid analysis of paracetamol in suppositories. The limits of detection and quantitation were calculated by statistical means and are relatively high. This is due to the fact that the detection wavelength of 220 nm was relatively insensitive compared to the wavelength of maximum absorbance of 243 nm for paracetamol in the microemulsion. Detection and separation of paracetamol and related substances was optimised using the same standard microemulsion with a gradient of (50/50, microemulsion/0.05%TFA) ramped up to 100% microemulsion in 8 min at a flow rate if 1.2 ml min$^{-1}$. Excellent resolution of paracetamol and five related substances was achieved in approximately 7.2 min. Although no reference method was carried out to compare retention times, the British Pharmacopoeia 2005 [14] does give the relative retentions times of paracetamol, 4-nitrophenol, 4-aminophenol and 4-chloroacetanilide which are compared in Table 2. It can be seen that the overall elution time of the four compounds is similar however the elution order of the compounds is different. In a study by Nageswara [13], separation of paracetamol and nine process impurities, which included the five compounds used here, was achieved in approximately 45 min using reversed-phase gradient HPLC. The last compound to elute was 4-chloroacetanilide, which eluted using gradient MELC in 5.3 min. Gradient MELC was shown to be a rapid method for paracetamol stability studies and offers the possibility of rapid determination of degradants and impurities in very hydrophobic formulations. This will be the subject of future method optimisation and validation studies.

Table 1
Validation data (paracetamol)

| | |
|---|---|
| Linearity | Range (mg ml$^{-1}$): 0.004–0.02; regression equation: $y = 74.681x + 0.009$; $R^2$: 0.9999; residual S.D. of the regression line ($\sigma$): 0.0052 |
| Accuracy ($n = 8$) | Recovery: 99.75%; S.D.: 1.299; R.S.D.: 1.3% |
| Precision | Repeatability ($n = 9$): R.S.D. = 0.70%; inter. precision ($n = 9$): R.S.D. = 0.84% |
| LOD | 0.0002 mg ml$^{-1}$ |
| LOQ | 0.0007 mg ml$^{-1}$ |
| Assay (% label claim) | MELC assay: 100.1%; reference method assay: 99.8% |

Table 2
Comparison of gradient MELC and BP 2005 retention times (min) for paracetamol and related substances

| | Gradient MELC | BP 2005 |
|---|---|---|
| Paracetamol | 1.1 | 4 |
| 4-Aminophenol | 2.8 | 0.8 |
| 4-Chloroacetanilide | 5.3 | 7 |
| 4-Nitrophenol | 7.2 | 3 |

## 8. Conclusion

An isocratic oil-in-water microemulsion liquid chromatographic method has been optimised and validated for the rapid determination of paracetamol in a suppository. The ability of O/W microemulsions to quickly solubilise highly hydrophobic sample matrices has been shown. This eliminated the need for sample pre-treatment and/or extraction steps before analysis and a significant reduction in analysis times for paracetamol suppositories was achieved, compared to a reference method. The same standard microemulsion, with gradient elution, was used to rapidly separate paracetamol and five of its related substances.

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