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ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 44 (2007) 137–143
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

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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Received 3 January 2007; received in revised form 16 February 2007; accepted 19 February 2007
Available online 23 February 2007

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.

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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doi:10.1016/j.jpba.2007.02.025

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^{TM}, 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