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Talanta 66 (2005) 229–235
Talanta
www.elsevier.com/locate/talanta

Selective spectrofluorimetric method for paracetamol determination through coumarinic compound formation

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Received 20 May 2004; received in revised form 10 November 2004; accepted 18 November 2004
Available online 25 December 2004

Abstract

A spectrofluorimetrical selective method was designed for determination of paracetamol in tablets. This important technique can be characterized by its sensitivity, simplicity, celerity and cheaper cost than current official methods. The employed methodology involves coumarinic compound formation obtained by reaction between paracetamol and ethylacetoacetate (EAA) in the presence of sulphuric acid as catalyst. The reaction product is highly fluorescent at 478 nm, being excited at 446 nm.

The linear concentration range of the application was 0.1–0.4 μg/ml of paracetamol and the detection limit was 57 ng/ml.

The influence of different variables was studied and optimized through chemometric techniques. Applying the above-mentioned method good results were obtained with regard to pharmaceutical formulations containing paracetamol. Therefore, it is relevant to suggest this profitable technique for medicament control analysis.

Keywords: Fluorescence; Paracetamol; Ethylacetoacetate; Coumarin

1. Introduction

Paracetamol or acetaminophen (*N*-(4-hydroxyphenyl) acetamide) (Scheme 1) is an effective alternative to the aspirin as an analgesic and antipyretic agent. Compared to aspirin, paracetamol's anti-inflammatory activity is considered weaker.

This substance can be characterized by its tolerance, its gastric indisposition absence and its free selling. Furthermore, acetaminophen is often self-prescribed, without medical control, to alleviate moderate pain, to calm fever, lumbar pain, migraine or non-specific indications [1].

Recent studies have shown that paracetamol is associated to hepatic toxicity and renal failure despite of its apparent innocuous character. Hepatic toxicity begins with plasma levels of paracetamol in the 120 μg/ml range 4 h after the ingestion and an acute damage is presented with plasmatic levels up to 200 μg/ml 4 h after the ingestion.

At normal therapeutic doses, paracetamol is metabolized very fast and completely by undergoing glucuronidation and sulfation to inactive metabolites that are eliminated in the urine. However, paracetamol higher doses produce toxic metabolite accumulation that causes hepatocyte death.

Approximately 0.01% of the US population and 0.02% of the Australian population are assessed in hospital each year because of paracetamol poisoning [2].

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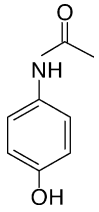
doi:10.1016/j.talanta.2004.11.015



Scheme 1.

Acetaminophen overdose is a frequent cause of fulminating hepatic failure in Europe and US [3].

Based on the aforementioned observations the main objective was to carry out the development of more efficient analytical techniques, destined to quality control of one of the medicaments more widely used.

Several analytical procedures are proposed for the determination of paracetamol in pharmaceutical products, e.g., titrimetric [4], colorimetric [5], UV–visible absorption [6], voltammetric [7], flow-injection systems (FIA) with colorimetric detection [8,9], Fourier transform infrared spectrometry [10], HPLC [6,11], micellar liquid chromatography [12] and many others.

Both the BP [6] and USP [11] recommend an HPLC method for the determination of paracetamol in pharmaceutical formulations, which requires high sophistication and very expensive cost of equipment.

The determination of paracetamol tablets can be carried out by direct ultraviolet absorption spectrophotometry (monograph in BP [6]), however, when formulated with other UV-absorbing substances such as excipients or other drugs, where spectral overlap is possible, separative techniques are necessary. The notorious advantages of the proposed methodology are the reduction of analytical costs and a very interesting alternative to those labs, which do not have such sophisticated equipments as required to carry out official techniques.

Many spectrofluorimetric methods have been proposed for the determination of paracetamol as single or as a mixture with other drugs in pharmaceutical formulations, such as indirect determination using Ce(IV) as an oxidant agent [13], reaction with fluorescamine [14], reaction with dansyl chloride [15], 1-nitroso-2-naphthol [16], potassium hexacyanoferate (III) [17], oxidation with 2,2′-dihydroxy-5,5′-diacetyldiaminebiphenyl [18]. However, many of these methods show low selectivity and interference with other drugs and excipients can be observed. This problematic situation does not appear with the proposed method for spectrofluorimetrical determination of paracetamol presented to this paper.

In this work, it is intended to develop a selective technique for paracetamol determination in pharmaceutical formulations, which can also contain other drugs without interference between them.

This methodology is based on reaction between paracetamol and ethylacetoacetate in presence of a dehydrating agent such as sulfuric acid producing a coumarinic compound, which is spectrofluorimetrically measured [19–22].

The developed method was applied to the analysis of four different commercial pharmaceutical tablets obtaining good results compared to those acquired through official methods, which involved the use of expensive equipment and reagents in routine analysis.

Finally, the importance of medicaments control is to guarantee the safety and the trust in pharmaceutical formulations, which are of common using and non-prescripted. Therefore, it is very relevant to emphasize that they may cause death due to its toxicity and absence control.

2. Experimental

2.1. Apparatus

All fluorescence measurements were made on a Shimadzu RF-5301 PC spectrofluorophotometer with excitation and emission band pass of 5 nm using 1.0 cm quartz cells.

A Beckman DU 520 UV–visible spectrometer with quartz cells of 10 mm path length for absorptiometric measurements was used. NMR spectra were obtained using a Bruker AC-200 spectrometer.

2.2. Reagents

The ethylacetoacetate (Anedra) labeled to contain 98% (v/v) was prepared as 2% (v/v) solution in absolute ethanol and should be freshly prepared. Sulfuric acid (Merck) labeled to contain 98% (v/v). Paracetamol was supplied by Novartis Lab, Argentina. All solvents used were HPLC grade and all other reagents employed were of analytical grade and were used without further purifications.

2.3. Preparation of the standard solution

An accurate mass 15 mg of weighted paracetamol was transferred into 100 ml volumetric flask and dissolved in absolute ethanol to obtain a standard solution of 1×10^{-3} mol l^{-1}.

A working standard paracetamol solution obtained by dilution with the same solvent to give final concentrations of 1×10^{-5} mol l^{-1}. From this solution, a series of dilutions were prepared in absolute ethanol to obtain a range of concentrations 7.5×10^{-7} to 3×10^{-6} M. The standard solution was protected from light. The solutions were stable for at least 1 week, if they had been stored in a dark place.

2.4. Construction of the calibration graphs

A set of volumetric flask aliquot solutions of working standard paracetamol were quantitatively transferred to each