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Capillary ultra performance liquid chromatography–tandem mass spectrometry analysis of tienilic acid metabolites in urine following intravenous administration to the rat

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ABSTRACT

Capillary scale (100 mm × 150 µm id) UPLC/MS/MS, performed using reversed-phase gradient chromatography on sub 2 µm particles, has been successfully employed for the characterization of the metabolites of the drug tienilic acid (TA) excreted via the urine following oral administration to the rat. The capillary LC system provided a significant increase (range ca. 11–33-fold) in sensitivity compared with a conventional 150 mm × 2.1 mm id UPLC system. An investigation of the effect of the injection volume and sample mass loading on the capillary column on the results obtained for both endogenous metabolites and TA was performed. This demonstrated that the injection of up to 2 µL of rat urine onto the system was permitted whilst still providing excellent chromatographic results and robustness. Qualitative analysis of the urine revealed the presence of TA itself and a total of 15 metabolites of the drug, including those resulting from biotransformations such as hydroxylation or conjugation. The capillary chromatography system was shown to be robust, and capable of providing comprehensive drug metabolite profiles from small format urine samples such as those obtained from preclinical studies in rodents.

1. Introduction

Rapid advances in column technology over the last decade, in particular the introduction of both sub 2 µm column packing materials, and the development of ever more powerful MS platforms, have significantly enhanced the capabilities of LC/MS. In the area of pharmaceutical research these advances have greatly facilitated the investigation of the metabolic fate and pharmacokinetics of drugs and other xenobiotics allowing these "DMPK" studies to be performed rapidly, efficiently and with greater sensitivity. There is however, an increasing trend to reduce the sample volumes taken in studies undertaken in both pre-clinical species and humans (volunteers and patients) for both practical and ethical reasons. One result of this is an increasing move towards the adoption of dried blood spot/micro sampling technologies for sample collection [1–4], particular with respect to rodents used in pre-clinical studies. In rodent DMPK studies the advantages of reductions in sample size can be profound in terms of both the improved quality of the data obtained and reductions in animal use. Thus, the collection of smaller samples allows e.g., multiple blood samples to be taken from individual animals, enabling complete pharmacokinetic profiles to be obtained from individual rats or mice, thereby reducing the overall animal numbers required to obtain this key data. Similarly, the advantages of microsampling are also obvious for monitoring patients and in paediatric studies. Microsampling is, of course, not a panacea, and the problems encountered include the obvious difficulty of the precise manipulation of small sample volumes as well as e.g., variability in sample composition between single drops of blood [5]. Similarly, variability in sample collection, application, and factors such as haematocrit values has been of concern in the implementation of blood spots for quantitative analysis [6].

Whilst conventional LC/MS/MS systems are usually quite capable of servicing the analytical requirements of drug discovery the potential of capillary scale and nano scale LC to provide a further option for the analysis of precious small volume samples is clear. Reduction of the column diameter from 2.1 or 1 mm to 150 µm results in a much more concentrated peak with a consequent improvement in MS detection sensitivity [7]. Whilst capillary LC (cLC), and nano scale LC, have clearly demonstrated increased in sensitivity compared to conventional

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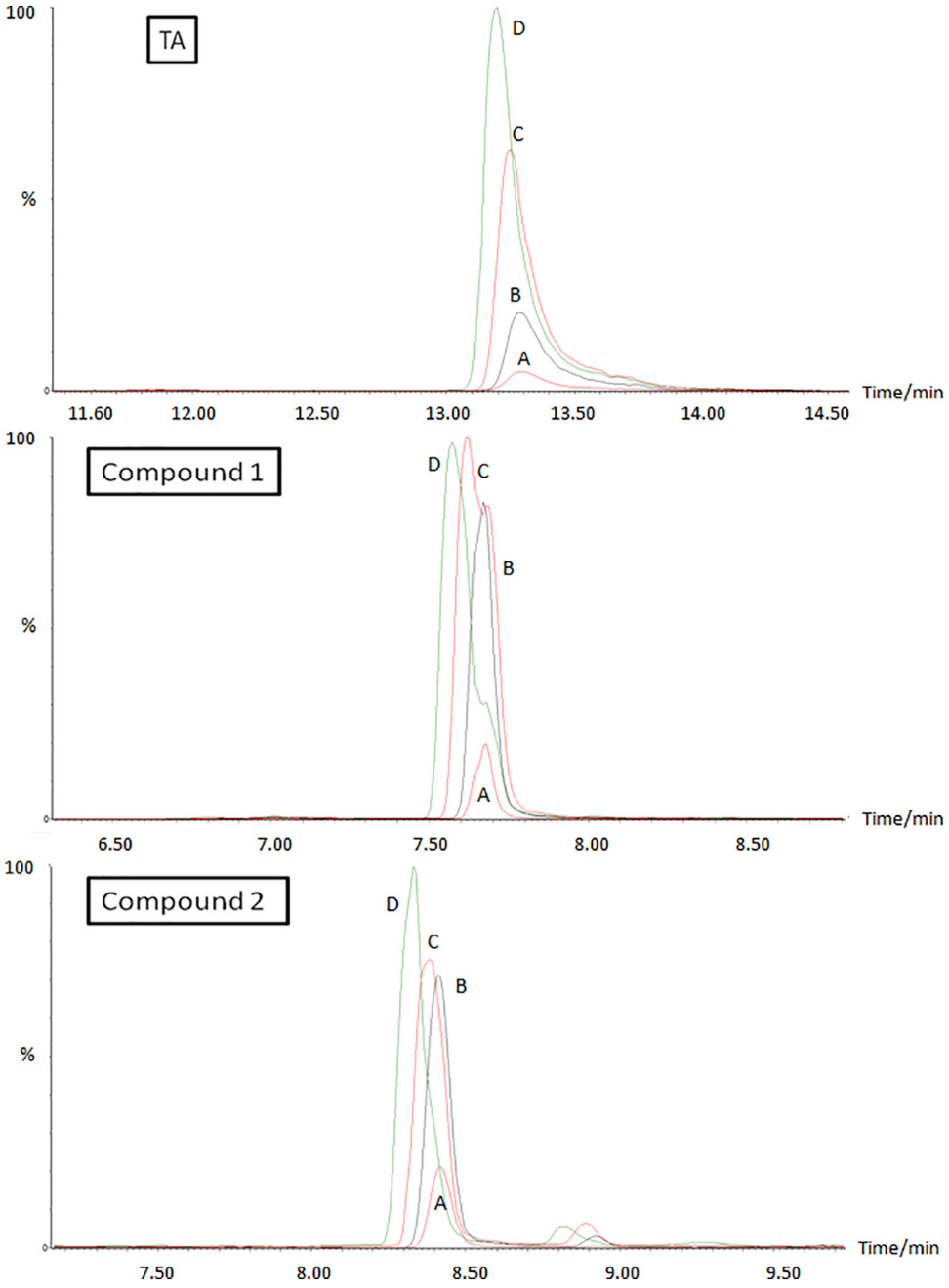

Fig. 1. Representative extracted ion chromatograms of tienilic acid and endogenous reference compounds 1 and 2 used for assessment of capillary LC performance at increasing injection volumes, 0.1 (A), 0.5 (B), 2.0 (C), 5.0 µL (D).

LC the lack of reliable systems to deliver this enhanced performance from specialist laboratories to the general chromatographic community has lagged behind. Even the introduction of commercial cLC systems in the late 1990's early 2000's [8–10] did not quite eliminate the need for the user to exercise skill to make the required connections to obtain the best performance from the system. This is because even small voids in column connections etc. have very deleterious effects on performance. Notwithstanding these limitations cLC systems have been used with some success in both proteomic and DMPK applications [11] including in the case of the latter quantitative drug bioanalysis and the identification of drug metabolites [12].

More recently systems where the column, connections and MS emitter/spray tip are combined in a single device have been introduced, greatly reducing/eliminating many of the problems previously associated with coupling cLC to MS [12]. Further, advances in manufacturing have enabled these devices to be constructed with sufficient mechanical strength to be used with the high back pressures produced when using columns packed with sub 2µm porous particle and capable of use in routine applications. Previously we have described the application of such a device for the investigation of drug metabolites produced in vitro [13] and for model drugs in dried blood spots and plasma samples [14] and drug metabolism studies in mouse plasma from liver humanized mice [15]. These, and other, advances in cLC have recently been reviewed [16]. Here we describe a further example of the use of an integrated ceramic-based micro-fluidic device for the determination of metabolite profiles in rat urine following the administration of the drug tienilic acid (TA).