Journal of Pharmaceutical and Biomedical Analysis 128 (2016) 106–110

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

# Are fluorine-rich pharmaceuticals lost by partition into fluorous phases?

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ARTICLE INFO

*Article history:*
Received 1 February 2016
Received in revised form 13 May 2016
Accepted 16 May 2016
Available online 17 May 2016

*Keywords:*
Fluorine-rich pharmaceuticals
Fluorous phase partition
Droplet microfluidics
Multiple injections in a single experimental run

ABSTRACT

The recently developed technology of droplet microfluidics has demonstrated great potential for many applications such as biochemical assay, high throughput screening, cell culture, directed evolution, and chemical synthesis. Intrigued by its capabilities for miniaturization, flexible manipulation, rapid reagent mixing and high throughput experimentation and analysis, the pharmaceutical industry has begun to investigate droplet microfluidic implementation in medicinal and process chemistry. Segmented by an immiscible secondary phase, usually perfluorinated oil, aqueous or organic droplets serve as individual micro-reactors without suffering cross-contamination. As many drug molecules contain fluorines, it is necessary to investigate whether such compounds will be preferentially extracted into the fluorous phase *via* fluorophilic solvation, which could lead to erroneous analytical results. In this work, we chose drugs with up to 10 fluorines to probe their partition into perfluorodecalin (PFD) from a variety of organic solvents. A fast and straightforward MISER (Multiple Injections in a Single Experimental Run) LC–MS method was applied to measure the loss of drug after mixing with PFD. We found that no significant partition occurred, with the concentration of drugs in the 'experimental' group measured as ±10% of the 'control' group. The RSD% of multiple injections is <5%. The finding was further validated by the conventional LC–MS approach.



## 1. Introduction

In recent years research methodologies for developing new pharmaceuticals have undergone profound changes, with an ongoing series of laboratory innovations leading to accelerated experimentation at ever decreasing scale and increasing throughput [1–3]. Droplet microfluidic technology shows considerable promise in this regard, utilizing liquid droplets compartmentalized by an immiscible fluid as nanoliter to picoliter reaction vessels. Its capability of performing high throughput drug screening have been reported [4–6], and hybridization with chemical synthesis is of considerable interest [7,8]. As droplet microfluidics advances to be implemented in drug discovery workflows, an understanding of the strengths and limitations is needed. Fluorocarbons are widely utilized as the 'carrier phase' in droplet microfluidics because it does not dissolve most compounds and is immiscible with most organic solvents [9]. However, whether highly fluorinated compounds are soluble in fluorocarbons is a concern. Indeed, organic molecules with long fluorinated tags to be solubilized by fluorocarbons is the cornerstone of fluorous phase technology [10]. We were concerned that since many drug molecules contain fluorines, with some containing multiple (*e.g.* anacetrapib has 10 F), such compounds might be lost by partition into the fluorous carrier phase. In order to test this hypothesis we utilized MISER (Multiple Injections in a Single Experimental Run) LC–MS technique to analyze a number of fluorine-containing drug molecules in a range of organic solvents mixing with perfluorodecalin (PFD) (Fig. 1). MISER has been reported for profiling of high throughput experimentation [1], catalysts screening [11], enantiopurity analysis [12] and quantitation of an active ingredient in food samples [13]. It is best suited for directly visualizing the signal variation of single component in multi-parallel experiments, especially when samples are relatively clean. Hence MISER was chosen to quickly find out if significant partition exists.

## 2. Experimental

### 2.1. Reagents and materials

Compound 1–5 (Fig. 2) containing up to 10 fluorines was selected for this study. Compound 1 was purchased from Combi-Blocks (San Diego, CA, USA). Compound 2 was purchased from

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http://dx.doi.org/10.1016/j.jpba.2016.05.025
0731-7085/

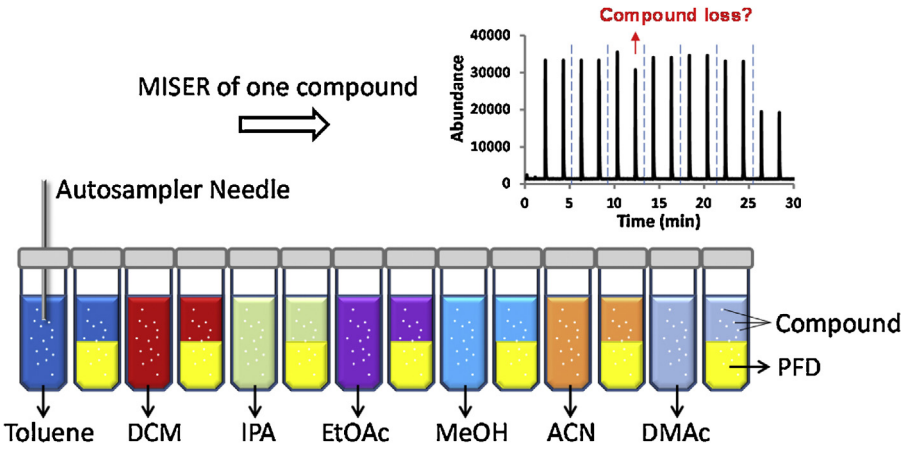
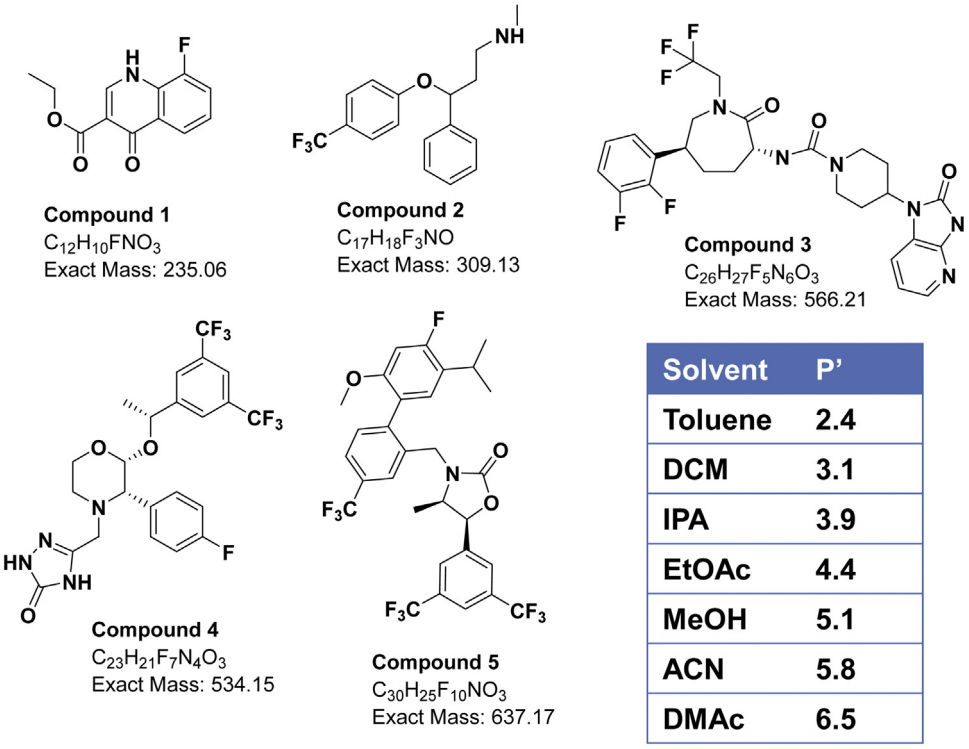



**Fig. 1.** Study design. Autosampler vials containing a test compound dissolved in different organic solvents are paired with a corresponding vial containing PFD. PFD is immiscible with all solvent used in this study and has higher density, therefore it migrates to the bottom of the vial after mixing. The MISER LC–MS system performed continuous injections of the organic layer from each vial and generated a 'misergrams' showing the extracted ion signal at the *m*/*z* of target analytes.




| Solvent | P' |
|---|---|
| Toluene | 2.4 |
| DCM | 3.1 |
| IPA | 3.9 |
| EtOAc | 4.4 |
| MeOH | 5.1 |
| ACN | 5.8 |
| DMAc | 6.5 |

**Fig. 2.** Structures of test compounds and the Polarity Index (P') of each solvent.

AstaTech (Bristol, PA, USA). Others were obtained from Merck Building Blocks Collection (Rahway, NJ, USA). The seven test organic solvents are: toluene, dichloromethane (DCM), isopropyl alcohol (IPA), ethyl acetate (EtOAc), methanol (MeOH), acetonitrile (ACN) and *N*,*N*-dimethylacetamide (DMAc). All solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA). The LC–MS mobile phase modifiers (ammonium formate and formic acid) were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

### 2.2. Instrumentation

The MISER LC–MS experiments were performed on an Agilent 1100 LC–MS system (Agilent Technologies, Santa Clara, CA, USA), which consists of a binary pump, a WPALS autosampler, a diode array detector and a 6120 Quadrupole LC/MS detector with the electrospray ionization (ESI) source. The separation was carried out on a 3.0 mm × 20 mm, 1.8 μm Agilent Zorbax SB-C18 column. Isocratic eluent consisting of 25% mobile phase A (10 mM ammonium formate, 0.15% formic acid aqueous solution, pH 3.5) and 75% mobile phase B (ACN) was generated by the binary pump for the fast separation at flow rate of 1 mL/min. The column temperature was kept at 40 °C. Flow injection mode was operated with 0.2 μL injection volume for every 2 min injection. The capillary voltage of ESI was +3.0 kV. The single quadrupole MS was operating in SIM (single ion monitoring) mode, extracting the *m*/*z* of all compounds simultaneously (*m*/*z* 236, 310, 535, 567, and 638).