Application Note: ANCCSMETFORMIN

# Analysis of Metformin on a Perfluorophenyl Stationary Phase by HPLC/UV

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**Key Words**

- Metformin
- Melamine
- Polar compounds
- Perfluorophenyl phase,
- Hypersil GOLD PFP
- Metformin (500 mg) tablets

## Introduction

Metformin [3-(diaminomethylidene)-1,1-dimethylguanidine] is an orally administered, anti-hyperglycaemic drug used for the treatment of Type 2 (non-insulin dependent) diabetes mellitus. Metformin improves glycaemic control by both enhancing insulin sensitivity, and, by decreasing intestinal absorption of glucose.

As a direct consequence of its high polarity (log P = - 2.64), metformin is difficult to retain on conventional stationary phases (e.g., C18 and C8) employed in reversed-phase chromatography [1]. Moreover, the strong interaction with polar media (e.g., silica and cyano) may mean that the biguanide drug cannot be eluted easily during its examination by normal phase chromatography [2, 3].

In view of the potential shortcomings associated with both of the above, alternative chromatographic approaches may be adopted for the separation of metformin and its related compounds (e.g., cyanoguanidine, melamine and 1-methylbiguanide), including ion-pair chromatography [4,5], hydrophilic interaction liquid chromatography (HILIC) [6,7], aqueous normal phase (ANP) chromatography [8,9] and ion-exchange (IEX) chromatography [10,11]. In fact, the US pharmacopeial procedure for the inspection of metformin specifies that separations must be performed in an ion-exchange manner using USP L9 column packings. However, ion-exchange techniques can suffer from some practical constraints. In particular, when used with MS, both the precipitation of salt and the suppression of ionisation of the analyte(s) under investigation can be observed.

Despite the existence of a variety of chemically bonded phases for the retention of hydrophilic molecules, perfluorinated phases (e.g., pentafluorophenyl) have attracted particular attention [12, 13]. Perfluorinated and fluorinated stationary phases have been found to exhibit novel selectivity for several classes of compounds and, in many instances, provide appealing alternatives to traditional C18 and C8 phases.

The purpose of this chromatographic study is to demonstrate the effectiveness of a perfluorophenyl stationary phase for the retention of metformin and its related compound melamine under typical reversed-phase conditions.

## Experimental details

### Chemicals and reagents

| | |
|---|---|
| Fisher Scientific Ammonium acetate (AR grade) | A/3440/50 |
| Fisher Scientific Ammonia solution (HPLC grade, SG – 0.88, 35 %) | A/3295/PB05 |
| Fisher Scientific Acetonitrile (HPLC grade) | A/0626/17 |
| Fisher Scientific Water (HPLC gradient grade) | W/0106/17 |
| Metformin (500 mg) tablets | |
| Metformin Hydrochloride (≥ 98 %) | |
| Melamine (≥ 99 %) | |

### Sample handling equipment

| | |
|---|---|
| Fisher Scientific Finnipipette F2 pipettor kit 10 µL – 100 µL, 100 µL – 1000 µL, 1 mL – 10 mL | PMP-020-220F |
| Fisher Scientific Finnitip pipette tips, 10 µL | PMP-107-110W |
| Fisher Scientific Finnitip pipette tips, 200 µL | PMP-107-600F |
| Fisher Scientific Finnitip pipette tips, 1000 µL | PMP-103-206K |
| Fisher Scientific Finnitip pipette tips, 10 mL | PMP-107-040R |
| Fisher Scientific Syringe filters (PVDF filter membrane, 30 mm O.D., 0.45 µm pores) | HCA-442-013Q |
| Thermo Scientific Borosilicate glass vials (2 mL, 12 mm x 32 mm) with 8 mm black screw cap fitted with a silicone/PTFE seal | 60180-600 |

### Sample Preparation

#### Analytical standards

Primary analytical standards of both metformin hydrochloride and melamine were prepared separately by the dissolution of approximately 0.01 g (weighed accurately) of reference material in water (10.0 mL). The concentration of each analyte was approximately 1000 µg/mL.

Thereafter, a mixed working standard was prepared by combining 1 part of each primary standard with 98 parts of mobile phase (90:10 (v/v) $NH_4OAc$ – MeCN). The concentration of each analyte in the working standard was approximately 10 µg/mL.

### Extraction of Metformin (500 mg) tablets

A number (x 4) of metformin tablets was ground to a powder using a mortar and pestle. Thereafter, a portion (approximately 120 mg) of the powder, containing a nominal quantity of about 100 mg of metformin hydrochloride, was transferred into a conical flask and subsequently brought into contact with water (100 mL). Following agitation of the mixture for 30 minutes at room temperature, a portion of the slurry (10.0 mL) was removed and allowed to pass through a 0.45 µm PVDF filter membrane. After discarding the first few millilitres of solution, the resultant, clear liquid was collected. Finally, the aqueous extract was diluted 100-fold by mixing 1 part of aqueous extract with 99 parts of mobile phase (90:10 (v/v) $NH_4OAc$ – MeCN).

### Instrumentation

Thermo Scientific HPLC system equipped with a PDA detector. Data were acquired and processed using ChromQuest 4.2 software.

### Chromatographic conditions

| | |
|---|---|
| Column - Thermo Scientific Hypersil GOLD PFP 5µm 150 mm x 4.6 mm | PN: 25405-154630 |
| Mobile phase | $NH_4OAc$ 20 mM, pH - 7.98 / MeCN (90:10 v/v) |
| Flow rate | 1.0 mL/min |
| Column temperature | 30 °C |
| UV detection | 233 nm |
| Injection volume | 5 µL |
| Run time | 5 minutes |
| Syringe flush | mobile phase |

## Results

Under the isocratic conditions adopted for this analysis, adequate retention and separation of these polar molecules can be accomplished in less than 5 minutes. The chromatographic performance of the pentafluorophenyl (PFP) phase during the analysis of a mixture of metformin and melamine is summarised in Table 1. Chromatographic data are matched with excellent precision. Comparative chromatograms of both a standard solution of the polar analytes and a solution derived from an aqueous extract of an amount of powdered 'Metformin 500 mg' tablets are shown in Figure 1.

This qualitative approach precludes any accurate determination of the content of metformin (and melamine) in the tablets. Despite the fact that a detailed mechanism accurately describing the retentive behaviour of the perfluorophenyl phase is still to be elucidated, it has been proposed that the interaction between the carbon-fluorine dipole (on the aromatic PFP ring) and the amino groups in the metformin molecule is primarily responsible for the observation of enhanced retention.

| | Retention time (min) | | Efficiency (USP Plates/m) | | Tailing factor (USP) | | Resolution (USP) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | %RSD | Mean | %RSD | Mean | %RSD | Mean | %RSD |
| Melamine | 2.101 | 0.09 | 71916 | 0.64 | 1.32 | 1.18 | - | - |
| Metformin | 3.322 | 0.23 | 51755 | 0.50 | 1.66 | 1.02 | 10.54 | 0.24 |

Table 1. Chromatographic performance of Hypersil GOLD® PFP (data derived from 10 replicate injections)

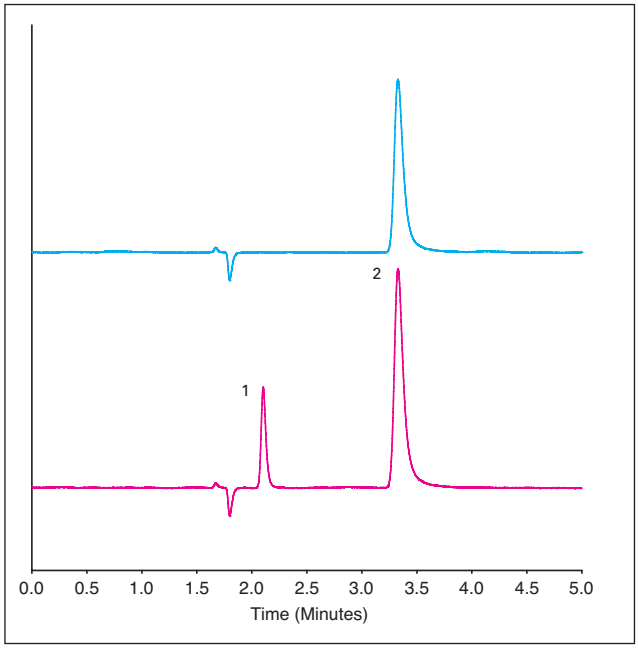


Figure 1. Comparative chromatographic profiles: Upper trace – aqueous extract of metformin (500 mg) tablets; lower trace – standard solution of metformin and melamine; 1. melamine, 2. metformin

## Conclusions

Perfluorophenyl bonded phases may be used for the successful retention of the hydrophilic molecules metformin and melamine. Moreover, good retention and separation can be achieved under typical reversed-phase conditions in less than five minutes.

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