An automated dual stage solid phase extraction procedure for 15 F2t-isoprostane (8-iso Prostaglandin2a) from BSA as lipid markers of oxidative stress

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Introduction

F2-isoprostanes are lipid markers of oxidative stress in animal host systems. F2-isoprostanes have been identified as biomarkers of cardiovascular disease, pulmonary disease, neurological disorders and various conditions related to the health and function of the kidneys and liver. Strategies to measure these analytes are typically laborious multi-step procedures that are plauged with low recovery sample preparation strategies leading to poor method precision. Targeting endogenous lipids as analytes of interest is challenging since modern solid phase extraction procedures (SPE) typically aim to remove lipids in favor of targeting small molecules. For these reasons, method optimization strategies are required to improve the efficacy of current testing protocols. In this work, the automation of an exisiting SPE procedure was implemented. A dual stage cleanup strategy was employed.

It is anticpated that this report will have impact in labs that provide testing platforms for population studies focused on lipids as markers of oxidative stress. This automation platform demonstrates an improvement in lab efficiency and data quality.

Analyte of Interest

The structure of the anlyte of interest is given in Figure 1. F2-isoprostanes are the product of free radical-catalyzed peroxidation of arachidonic acid. The estimated analyte pKa ~ 4-5.

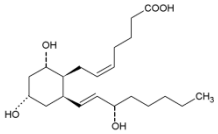

Figure 1: *Structure of the analyte of interest*

Experimental Procedure

The sample preparation method protocol was previously published in Free Radical Biology and Medicine vol. 59 pp 36-44. This report details the automation of the dual stage SPE methods. For the full details of this analytical procedure, please reference this work.

Automation was achieved using a RapidTrace+ system with automated data acquisition software. It was determined that there were several observed advantages to this approach vs offline manual preparation including laboratory efficiency and figures of merit describing data quality. The SPE platform was 3 mL cartridges supporting 500 mg sorbent beds.

REAGENTS

The reagents methanol, acetonitrile, heptane, ethyl acetate, and chloroform, were all acquired from Fisher Scientific (HPLC grade). Standards (8-iso Prostaglandin F2α and 8-iso Prostaglandin F2α-d4) were acquired from Cayman Chemical (Ann Arbor, MI).

Gas Chromatography - Mass Spectrometry

Detection of the target analytes was optimized using a Shimadzu gas chromatography system (QP-2010) coupled to a quadrupole mass selective detector. The GC column was an Rtx-5 30 meter, 0.32 mm ID, 0.25 µm df, Restek Corp, Bellefonte, PA. The ion source was operated in the negative ion chemical ionization mode. The injection volume was 2 µl. Injection was splitless. The carrier gas was helium. The selected ion monitoring (SIM) parameters relevant to this study are detailed in Table 1. The reagent gas was methane.

Table 1*: SIM parameters for GC-MS analysis*

Analyte	Mass-to-charge (m/z)
15-F2t-IsoP	569
[2 H4]-15-F2t-IsoP	573

Automation of the solid phase extraction procedure

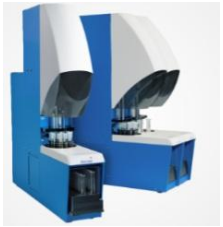

Figure 2: *The RapidTrace+ automated SPE system*

Automation of the dual stage method was achieved using a RapidTrace workstation (Figure 2).. The lines were purged prior to running the method program. Preliminary data comparing the automation of this method versus a manual prep is detailed in Figure 3&4. The encouraging results prompted further optimization of load volumes and flow rates. The optimized method program parameters are detailed in Tables 2a and b.

Results- Relative recovery and repeatability

The relative recovery for the extraction of the analyte from a formulated matrix of normal saline containing BSA was determined using the ratio of the analyte peak area/internal standard peak area of the pre-extraction fortified matrix specimens compared against the extracted matrix fortified post-extraction. The concentration level was 1 ng/mL. The performance for 5 replicates is given in Figure 5. **Typical recovery values were ~60-70% in comparison with typical recovery √values obtained by manual preparation were < 50%.**The improved relative recovery may be attributed to improved control of flow rate. The low recovery observed in the first replicate was attributed to the relatively high peak area response on the internal standard. A repeatability study for n = 5 replicates comparing sample prep methods with and without automation is given in Figure5. The fortified BSA concentration level was 2 ng/mL.

Table 2: *RapidTrace+ method dual stage method parameters a) C18 b) Si*

a) C18 cartridge 500 mg 3mL

C18 Step #	Step	Source	Output	Vol	mL/ min
1	Purge-cannula	EtOAc: heptane	Cannula	5	30
2	Purge-cannula	MeOH	Cannula	5	30
3	Purge-cannula	heptane	Cannula	5	30
4	Purge-cannula	pH 3, 0.001N HCl	Cannula	5	30
5	Condition	MeOH	Waste	5	30
6	Condition	pH 3, 0.001N HCl	Waste	5	15
7	Condition	pH 3, 0.001N HCl	Waste	2	15
8	Load	Sample	Sample	5	1.5
9	Load	Sample	Sample	5	1.5
10	Wash	heptane	Waste	5	5
11	Wash	heptane	Waste	5	5
12	Collect	EtOAc:heptane	Fract 1	5	2
13	Collect	EtOAc:heptane	Fract 1	5	2

*These extracts are dried over sodium sulfate and decanted.

b) Si cartridge 500 mg 3mL

Si Step #	Step	Source	Output	Volume	mL/ min
1	Purge	EtOAc: methanol	cannula	4	30
2	Purge	EtOAc	cannula	4	30
3	Condition	EtOAc	waste 1	5	30
4	Load	Sample	waste 1	5	1.5
5	Load	Sample	waste 1	5	1.5
6	Wash	EtOAc	waste 1	5	2.5
7	Collect	EtOAc: methanol	Fract 1	5	2

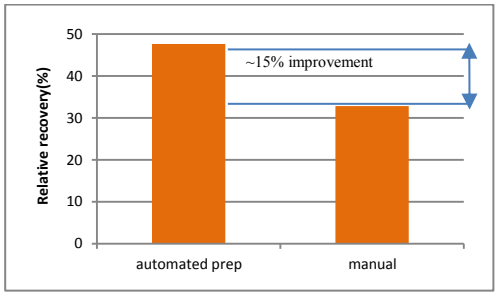

Figure 3: *Preliminary data from method comparing automated methodon RapidTrace+ (not optimized) in BSA versus manual prep (n=5)*

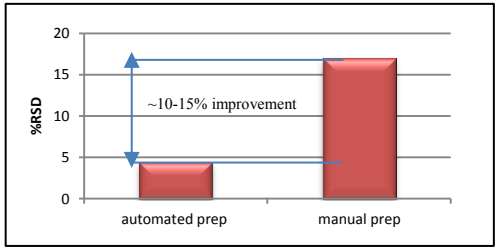

Figure 4: *Repeatability (%RSD) comparison of extracted BSA samples prepared with and without automation (n = 5)*

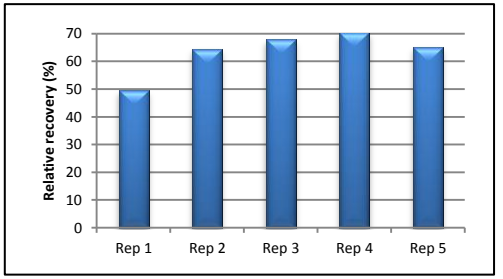

Figure 5: Relative recovery of F2 isoprostane from serum.

Conclusions

Automation of the existing sample preparation method offers the following advantages:

- Increased lab efficiency
- Reduced cost in technician bench time
- Improved relative recovery and method precision

Part Number: P59