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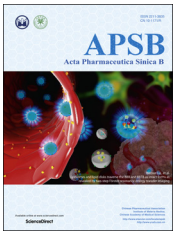

ORIGINAL ARTICLE

Separation and simultaneous quantitation of PGF2α and its epimer 8-*iso*-PGF2α using modifier-assisted differential mobility spectrometry tandem mass spectrometry

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Abstract Because many therapeutic agents are contaminated by epimeric impurities or form epimers as a result of metabolism, analytical tools capable of determining epimers are increasingly in demand. This article is a proof-of-principle report of a novel DMS–MS/MS method to separate and simultaneously quantify epimers, taking PGF2α and its 8-epimer, 8-*iso*-PGF2α, as an example. Good accuracy and precision were achieved in the range of 10–500 ng/mL with a run time of only 1.5 min. Isopropanol as organic modifier facilitated a good combination of sensitivity and separation. The method is the first example of the quantitation of epimers without chromatographic separation.

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1. Introduction

Epimers are a class of stereoisomers containing more than one chiral center that differ in configuration at one stereogenic center only[1]. Unlike structural and geometric isomers, epimers often present very similar physical and chemical properties. Therefore, the selection of a suitable analytical method to separate and quantify epimers poses a significant challenge.

To date, a number of techniques have been developed to separate and simultaneously quantify epimers including high performance liquid chromatography (HPLC)[2], capillary electrophoresis (CE)[3], capillary gas chromatography[4], liquid chromatography–mass spectrometry (LC–MS)[5] and liquid chromatography tandem mass spectrometry (LC–MS/MS)[6]. In recent years, assay of epimers has been mainly based on LC–MS/MS which can be considered to involve three-stages of selectivity *viz* the chromatographic separation, the selection of a precursor ion in quadrupole 1 (Q1) and the selection of one or more product ions in quadrupole 3 (Q3). When two epimers possess the same precursor ion and fragment ions, chromatographic separation is a *sine qua non* of their simultaneous determination.

As with enantiomers, epimers have the same molecular weight and often give the same fragment ions in detection by tandem mass spectrometry, so their separation is often necessary for their quantitation. In some cases, HPLC is adequate for this purpose but retention times are often long making such assays of limited value for high throughput analysis. In addition, analysis of many small molecules and peptides by liquid chromatography tandem mass spectrometry (LC–MS/MS) is limited by inadequate fragmentation with associated interference or fragmentation to unstable product ions. Both of these limitations can be overcome to varying extents using modifier-assisted differential mobility spectrometry tandem mass spectrometry (DMS–MS/MS) in which ions are separated in a DMS cell based on their mobility in an electric field.

In 2007, a new separation technique called differential mobility spectrometry (DMS) was coupled to tandem mass spectrometry and a DMS–MS/MS instrument released commercially[7]. Separation in DMS occurs between the parallel plates of a drift cell where ions produced in an ionization source are separated on the basis of their drift times in a fixed electrical field. Ions are carried axially along the gap between the plates by a flow of gas (the transport gas, nitrogen) and displaced radially toward one or other plate by the application of a radio frequency waveform, the amplitude of which is called the separation voltage (SV). Most ions are neutralized before reaching the exit but, through application of a DC voltage to the plates called the compensation voltage (CoV), target ions can be steered into the central axis of the gap for detection at the exit. Each ion has a unique value of CoV for a particular value of SV that allows a continuous beam of that ion to pass to the detector for analysis.

Separation in DMS depends on differences in the dipole moment of ions which is related to their size, charge and spatial structure. It is also due to the difference in migration rates of ions in the high and low electric fields imposed by the SV. Resolution and peak capacity can also be enhanced by addition of an organic modifier to the transport gas. This is because the modifier enhances the formation of clusters which magnifies the low- and high-field mobility difference and changes the CoV[8]. Being a post-ionization technique, DMS is characterized by very short run times and provides the possibility of very high-throughput analysis. It also has the ability to reduce interference from difficult-to-separate co-eluting contaminants and from background noise.

Recently, DMS–MS has gained traction in the analysis of complex mixtures of isomers. Examples include its application (Qtrap) to determining unsaturated triacylglycerol regioisomers extracted from adipose tissue[9], its use (QqLIT) in the analysis of isobaric and isomeric structural isomers of $C_8H_{16}O_2$[10] and its application in the determination of nicotine and its isomer anabasine[11]. In all these analyses, different MRM channels monitored transitions from the same parent ion to different product ions and DMS served solely to reduce interference.

In order to explore the capability of DMS–MS/MS to separate and quantify hard-to-separate isobaric compounds, we selected a mixture of prostaglandin F2α (PGF2α) and its 8-epimer, 8-*iso*-PGF2α, as our research target. PGF2α, marketed as Dinoprost, is used to induce abortion in humans[12] and to improve conception rates in dairy cattle undergoing artificial insemination[13,14]. More importantly, PGF2α and 8-*iso*-PGF2α are both involved in some severe acute and chronic inflammatory conditions and 8-*iso*-PGF2α is an oxidative stress biomarker that appears to be elevated in syndromes such as vascular reperfusion injury, paracetamol poisoning and liver cirrhosis[15,16]. Prasain et al.[17] developed an LC–MS/MS method to determine PGF2α and 8-*iso*-PGF2α but the total run time was 19 min. This paper reports the successful application of modifier-assisted DMS–MS/MS to the separation and quantitation of these epimers in a run time of only 1.5 min.

2. Experimental

2.1. Reagents and chemicals

PGF2α ($C_{20}H_{34}O_5$, >99%) and 8-*iso*-PGF2α ($C_{20}H_{34}O_5$, >99%) (Fig. 1) were purchased from Santa Cruz Biotechnology (Dallas, Texas, USA). Acetonitrile (HPLC-grade) was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Distilled water was prepared from demineralized water. Solid phase extraction (SPE) cartridges were purchased from Waters (USA) for Oasis MAX and Agilent Technologies (USA) for Bond Elut DEA and Bond Elut C18.

2.2. Standards and quality control (QC) solutions

Stock solutions (100 µg/mL) of PGF2α and 8-*iso*-PGF2α were diluted with acetonitrile:water (50:50, *v/v*) to produce series of standard solutions (10, 30, 50, 100, 300 and 500 ng/mL) and QC solutions (10, 30, 100 and 400 ng/mL).

2.3. DMS–MS/MS conditions

DMS–MS/MS was carried out on an AB Sciex 6500 QTRAP MS/MS system equipped with a SelexIon® DMS cell (Concord, Ontario, Canada) mounted after the ionization source. Electrospray ionization (ESI) in the negative ion mode was performed by the Turbo-V ESI source set at 4500 V and 500 °C. Nitrogen was used as nebulizer gas (50 units), heater gas (50 units), curtain gas (20 units) and collision gas (medium). Declustering potential and collision energy were set to −40 and −28 eV, respectively. Detection of PGF2α and its 8-epimer was by multiple reaction

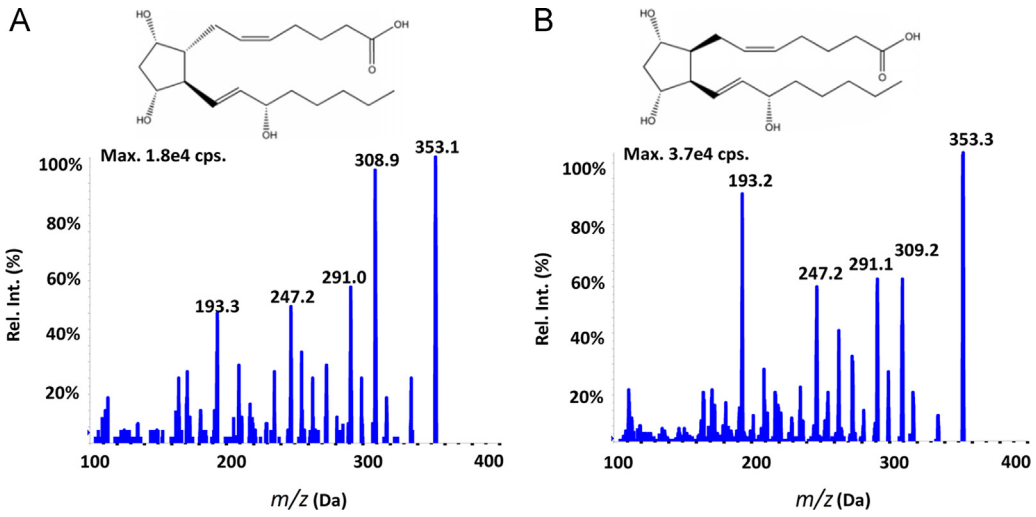

Figure 1 The structures and mass spectra of (A) PGF2α and (B) 8-*iso*-PGF2α.

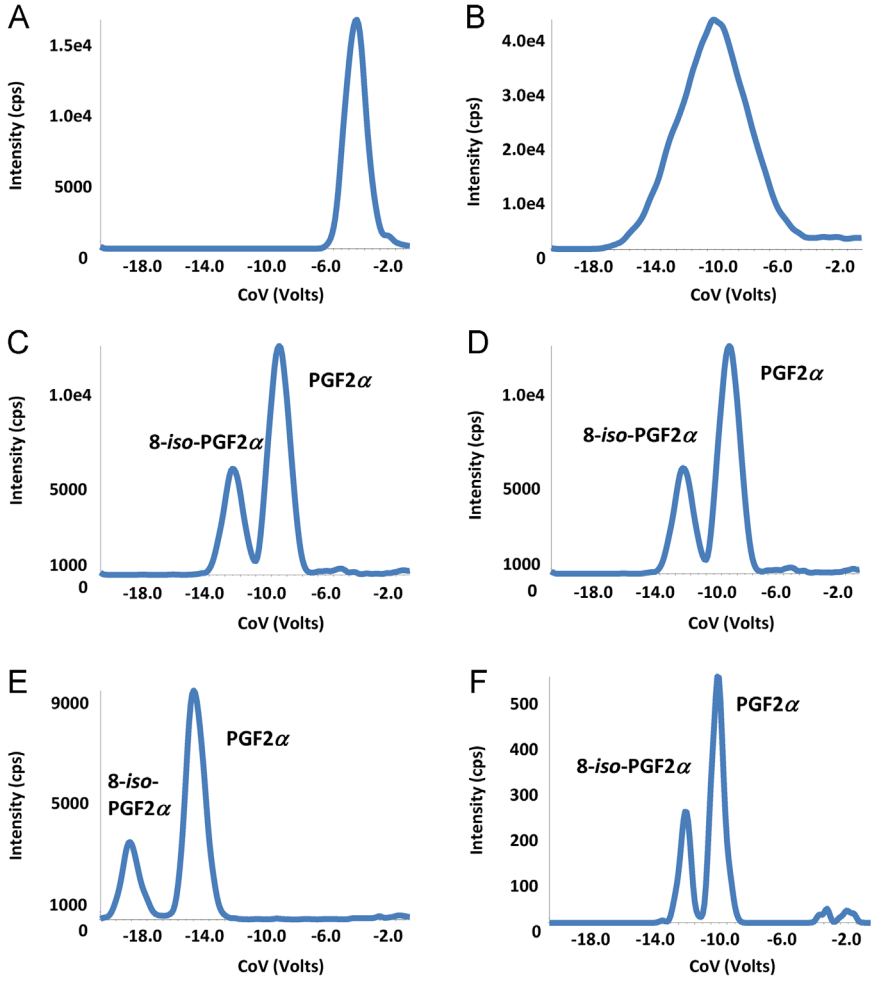

Figure 2 DMS ionograms illustrating optimization of DMS parameters [separation voltage (SV), resolution enhancement (DR) and DMS cell temperature (DT)] for the simultaneous determination of PGF2α and 8-*iso*-PGF2α using DT 150 °C: (A–C) Effect of SV (A) 2000 V , (B) 3000 V and (C) 3500 V using isopropanol as organic modifier, DR 20 psi; (D–F) effect of DR (D) 0 psi, (E) 20 psi and (F) 40 psi using isopropanol as organic modifier, SV 3000 V.

monitoring (MRM) of the transition at *m/z* 353.4→309.1. Data acquisition and integration were controlled by Analyst Software 1.6.2 (AB Sciex, Ontario, Canada).

Samples were introduced by the automatic sampler of an H class ultrahigh performance LC system (Perkin Elmer, Waldbronn, Germany) into acetonitrile: water (50:50, *v/v*) delivered at a flow

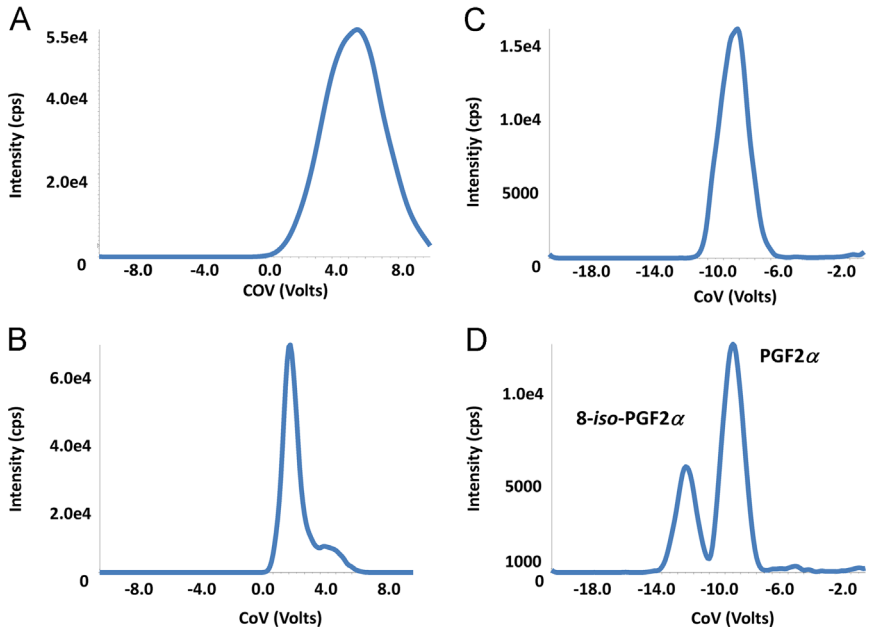

Figure 3 DMS ionograms illustrating the effect of organic modifier (A) no modifier (B) methanol (C) acetonitrile (D) isopropanol using SV 3000 V, DR 20 psi.

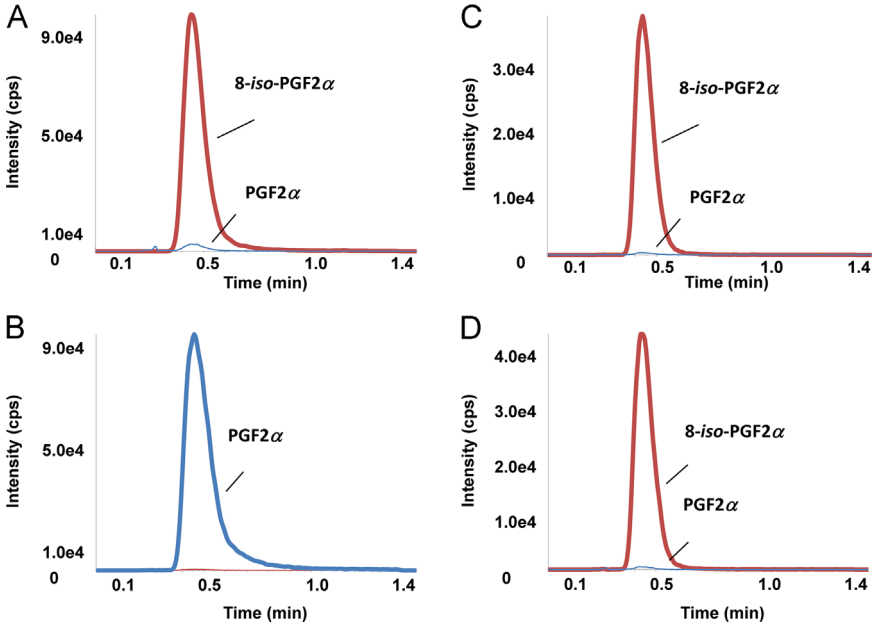

Figure 4 DMS ionograms illustrating investigation of the interconversion of PGF2α and 8-*iso*-PGF2α using DT 150 °C: (A) 8-*iso*-PGF2α with SV 3500 V, DR 20 psi; (B) PGF2α with SV 3500 V, DR 20 psi; (C) 8-*iso*-PGF2α with SV 3600 V, DR 30 psi; (D) 8-*iso*-PGF2α with SV 3500 V, DR 30 psi.

rate of 100 µL/min. Although chromatography was not involved in the assay, a C18 guard column (Phenomenex, Utrecht, the Netherlands) was positioned to prevent contaminants and micro-particulates entering the mass spectrometer and help minimize peak dispersion.

The DMS cell temperature (DT) and offset were set to 150 °C and 3.00 respectively. SV was 3000 V and CoVs were −8 and −11.6 V for PGF2α and 8-*iso*-PGF2α, respectively. Isopropanol was used as organic modifier and introduced into the transport gas at a flow rate of 250 µL/min. DMS resolution enhancement (DR) gas (nitrogen) was set at 20 psi.

2.4. *Assay validation*

Linearity was assessed by construction of calibration curves in triplicate and linear least squares analysis. Intra- and inter-day precision and accuracy were determined by assay of six replicate

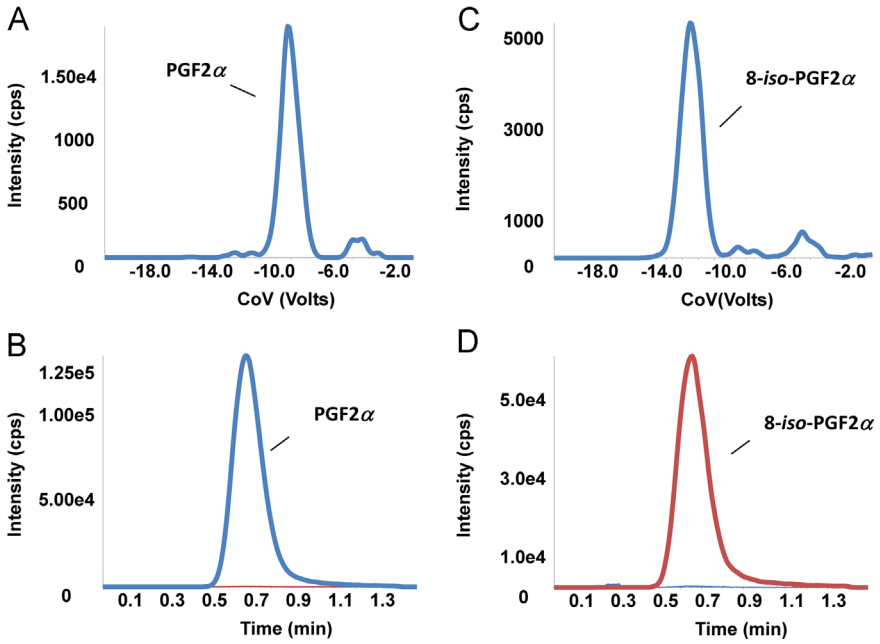

Figure 5 DMS ionograms and traces respectively of (A and B) PGF2α and (C and D) 8-*iso*-PGF2α using optimum conditions of SV 3500 V, DR 20 psi, DT 150 °C and introduction of isopropanol at 250 µL/min.

QC solutions on a single day and duplicating the experiments on three consecutive days. The stability of the two epimers was assessed by analyzing three replicate QC solutions after storage at room temperature for 4 h and at 4 °C in the autosampler for 4 h.

3. Results and discussion

3.1. MS/MS parameters

Examination of the product ion spectra of PGF2α and 8-*iso*-PGF2α showed they have exactly the same parent and product ions. Of the MRM transitions from the parent ion at *m/z* 353.4 to product ions at *m/z* 309.1, 291.2 and 193.3, the transition at *m/z* 353.4→309.1 gave the highest intensity and was selected for quantitation.

3.2. Optimization of DMS separation

In optimizing DMS parameters (Fig. 2), it was found that for SV < 3000 V (Fig. 2A), the two epimers could not be separated. Separation increased with increasing SV (Fig. 2B) but for SV > 3000 V (Fig. 2C) the intensity of the signals markedly decreased. In optimizing DR (the pressure of gas flowing in opposition to the transport gas), it was found that for DR < 10 psi (Fig. 2D) the two epimers shared the same CoV and could not be separated. Only when DR was increased to 20 psi (Fig. 2E) could separation be achieved consistent with the fact that increasing DR helps to narrow the CoV channel and contributes to a sharper peak. However, at DR > 20 psi (Fig. 2F), the signal intensity decreased greatly so that a value of 20 psi was selected. In choosing the organic modifier, methanol, acetonitrile and isopropanol were evaluated (Fig. 3B–D respectively) and all were found to shift the CoV towards more negative values. Isopropanol gave the best separation and was selected as organic modifier. DT was found to have little influence on separation and the low value of 150 °C was found to be acceptable.

Table 1 Least squares regression analysis of calibration curves of PGF2α and 8-*iso*-PGF2α on three different days.

Compd.	Least squares equation	*R*
8-*iso*-PGF2α	$y = 7.55e^3x - 2.58e^4$	0.9975
	$y = 7.81e^3x - 2.27e^4$	0.9990
	$y = 7.37e^3x - 1.09e^4$	0.9995
PGF2α	$y = 3.00e^3x - 1.20e^4$	0.9982
	$y = 3.06e^3x - 7.47e^3$	0.9993
	$y = 2.97e^3x - 7.84e^3$	0.9971

Previous work in our laboratory suggested the epimers could interconvert during analysis by DMS–MS/MS. In investigating this phenomenon, it was observed that when opening two CoV channels and injecting 8-*iso*-PGF2α solution only about 3% was converted to PGF2α (Fig. 4A); when injecting PGF2α, conversion in the opposite direction was negligible (Fig. 4B). The effects of changing SV and DR on the interconversion were examined. Comparing Fig. 4C and D shows that SV had a minimal effect on interconversion while comparing Fig. 4A and D shows that increasing DR results in less interconversion. In terms of the effect of the organic modifier, it was found that using a modifier reduced epimer interconversion and enhanced separation (Fig. 3).

Under the optimum analytical conditions of SV 3500 V, DR 20 psi, DT 150 °C and introduction of isopropanol at 250 µL/min, DMS traces (Fig. 5) confirmed that the assay was free of any interconversion of the epimers.

3.3. Assay validation

The assay was linear in the range 10–500 ng/mL for both PGF2α and 8-*iso*-PGF2α (Table 1). Intra- and inter-day precision and

Table 2 Accuracy and precision for the determination of PGF2α and 8-*iso*-PGF2α (data are based on assay of six replicate QC samples on three different days).

Compd.	Nominal conc. (ng/mL)	Mean found conc. (ng/mL)	Accuracy (RE%)	Precision (RSD%)	
				Intra-day	Inter-day
8-*iso*-PGF2α	10 (LLOQ)	11.0	8.17	4.88	–
	30	28.9	−3.56	4.32	6.61
	100	95.1	−4.89	3.66	13.1
	400	389	−2.83	4.18	3.31
PGF2α	10 (LLOQ)	11.0	8.83	2.89	–
	30	29.0	−3.44	3.85	13.5
	100	96.3	−3.66	3.09	10.9
	400	387	−3.35	4.50	2.13

–Not applicable.

Table 3 Stability of PGF2α and 8-*iso*-PGF2α under various storage conditions.

Compd.	Concentration (ng/mL)	Room temperature for 4 h [mean (RSD%)]	Autosampler 4 °C for 4 h [mean (RSD%)]
8-*iso*-PGF2α	30	32.6 (3.08)	30.1 (2.52)
	100	96.8 (1.35)	93.5 (6.48)
	400	354 (1.45)	371 (4.73)
PGF2α	30	31.6 (3.48)	30.6 (4.21)
	100	99.6 (1.24)	97.4 (1.44)
	400	363 (2.65)	373 (1.48)

accuracy were satisfactory (Table 2) and both epimers were stable on storage for 4 h at room temperature and for 4 h at 4 °C in the autosampler (Table 3).

3.4. *Application to urine samples*

An attempt was made to apply the assay to spiked urine samples. Sample preparation by SPE was evaluated using three types of SPE columns *viz* Bond Elut DEA (Agilent), Bond Elut C18 (Agilent) and Oasis MAX (Waters). Protein precipitation using different solvents was also examined. In all cases signal intensity was low and chromatographic peak shape abnormal to such an extent that concentrations of epimers could not be calculated. We maintain this is due to strong matrix effects leading us to conclude that while DMS provides an alternative strategy to chromatography in the analysis of hard-to-separate compounds, its application to biological samples demands good sample preparation or its use in conjunction with HPLC[18].

4. Conclusions

This is the first report of the separation and simultaneous quantitation of epimers by DMS–MS/MS, using PGF2α and 8-*iso*-PGF2α as a research target. Isopropanol as organic modifier facilitated a good combination of separation and sensitivity. Detection of both epimers was linear in the range 10–500 ng/mL with good accuracy and precision. The retention time was only 0.65 min for both analytes and the short run time of 1.5 min makes the assay suitable for very high-throughput analysis. The method illustrates the power of DMS to analyze hard-to-separate isobaric compounds.

Acknowledgments

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