Journal of Chromatography B, 1009-1010 (2016) 80–86

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Determination of four sulfated vitamin D compounds in human biological fluids by liquid chromatography–tandem mass spectrometry

Fabio P. Gomes, P. Nicholas Shaw, Amitha K. Hewavitharana *

School of Pharmacy, The University of Queensland, Queensland 4072, Australia

ARTICLE INFO

Article history:
Received 19 October 2015
Received in revised form 4 December 2015
Accepted 7 December 2015
Available online 9 December 2015

Keywords:
Vitamin D sulfate
Water-soluble vitamin D
Breastmilk
Human serum
Electrospray ionisation

ABSTRACT

The determination of both the water-soluble and lipid-soluble vitamin D compounds in human biological fluids is necessary to illuminate potentially significant biochemical mechanisms. The lack of analytical methods to quantify the water-soluble forms precludes studies on their role and biological functions; currently available liquid chromatography–tandem mass spectrometry (LC–MS/MS) methods are able to determine only a single sulfated form of Vitamin D. We describe here a highly sensitive and specific LC–MS/MS method for the quantification of four sulfated forms of vitamin D: vitamins D_2- and D_3-sulfate (D_2-S and D_3-S) and 25-hydroxyvitamin D_2- and D_3-sulfate (25(OH)D_2-S and 25(OH)D_3-S). A comparative evaluation showed that the ionization efficiencies of underivatized forms in negative ion mode electrospray ionisation (ESI) are superior to those of the derivatized (using 4-phenyl-l,2,4-triazoline-3,5-dione (PTAD)) forms in positive ion mode ESI. Separation was optimised to minimise co-elution with endogenous matrix compounds, thereby reducing ion suppression/enhancement effects. Isotopically labelled analogues of each compound were used as internal standards to correct for ion suppression/enhancement effects. The method was validated and then applied for the analysis of breastmilk and human serum. The detection limits, repeatability standard deviations, and recoveries ranged from 0.20 to 0.28 fmol, 2.8 to 10.2%, and 81.1 to 102%, respectively.

1. Introduction

It has been commonly accepted that vitamin D has a much wider role to play in the human organism rather than just its role in the homeostasis of healthy bone tissues. A growing body of evidences has been provided on the importance of this vitamin, in reducing the risk of hypertension, common cancers, diabetes mellitus, autoimmune and cardiovascular disorders [1,2]. Despite these findings, vitamin D deficiency still remains a commonly encountered health issue, affecting many individuals across the world [3].

While both fat- and water-soluble forms of vitamin D have been reported in diverse biological fluids, clinical and nutritional attentions have been primarily given to the fat-soluble forms [3,4]. Therefore, clinical studies on water-soluble Vitamin D compounds are lacking. It has been suggested that the water-soluble forms (sulfate conjugates) of vitamin D have potencies similar to those of the fat-soluble compounds [5]. Although the biosynthesis of the sulfates is unclear [6], a study has shown that vitamin D is not readily sulfated in man, indicating its formation from a conjugated precursor is possible [7]. The presence of 7-dehydrocholesterol-sulfate (7-DHC-S) has been reported in human and rat skin tissue, confirming the existence of a precursor for vitamin D_3-sulfate (D_3-S) [6]. While controversy exists regarding the specific actions and biological roles [8], it has been reported that vitamin D-sulfate (D_3-S), when orally administered in high doses, increases calcium transportation in young rats [9].

25-Hydroxyvitamin D_3-sulfate (25(OH)D_3-S) is a major circulating form of vitamin D and its levels in human blood may exceed those of the non-sulfated form, 25(OH)D_3 [6,7], the most commonly measured form of vitamin D to determine vitamin D status [3]. Clearly, the measurement of 25(OH)D_3-S in human blood is likely to be important in the assessment of vitamin D status. 25(OH)D_3-S could be considered a storage form of non-sulfated D_3, as hydrolysis of the conjugate might take place *in vivo* [4,6].

Vitamin D_2-sulfate (D_2-S) has been detected in chicken tissues and in rabbit urine [9,10] and the biosynthesis of D_2-S has been achieved *in vitro* [10]. It has also been demonstrated that D_2-S

* Corresponding author. Fax: +61 7 334 61999.
E-mail address: a.hewavitharana@pharmacy.uq.edu.au (A.K. Hewavitharana).

http://dx.doi.org/10.1016/j.jchromb.2015.12.014
1570-0232/

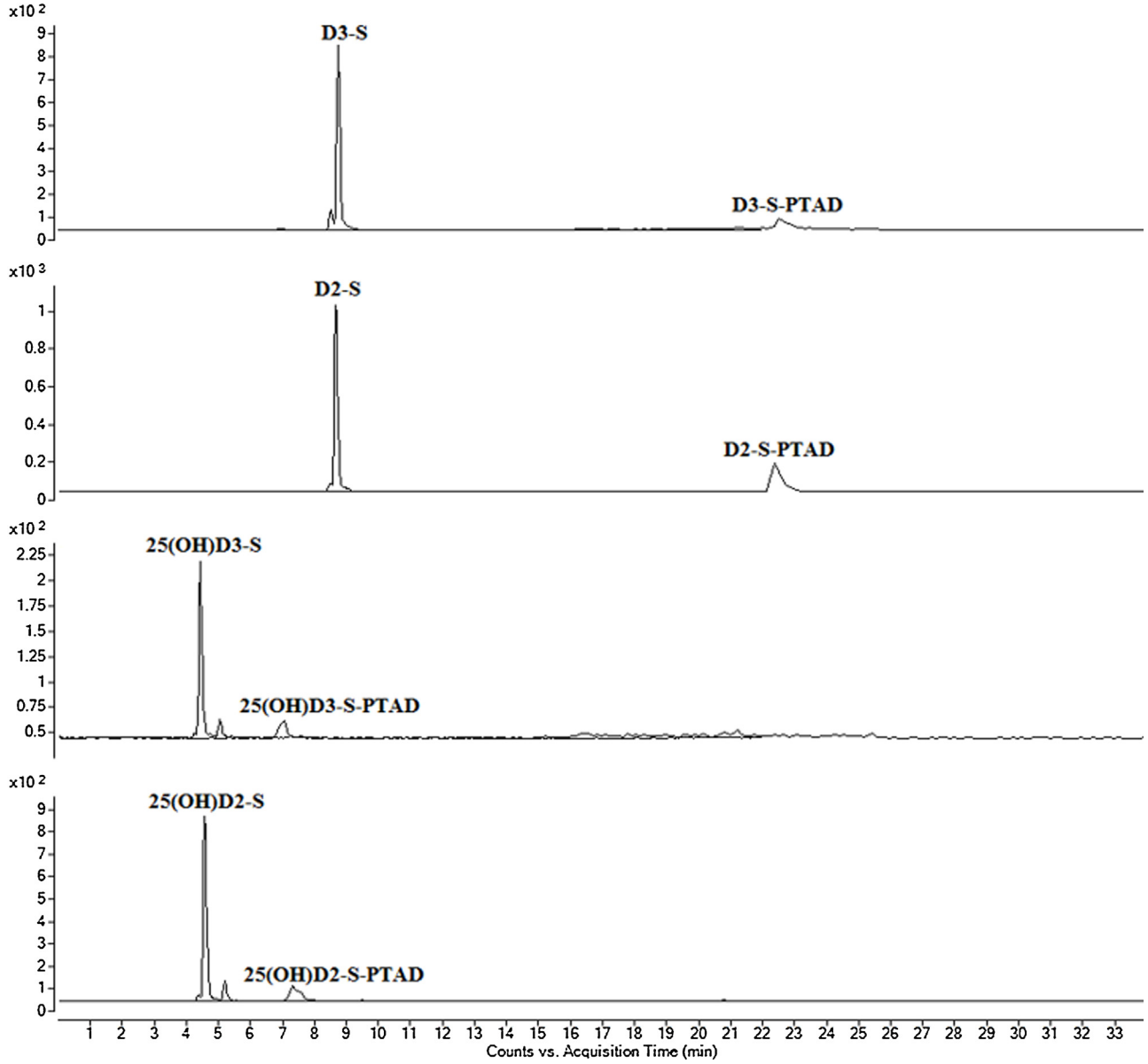

Fig. 1. Overlapped chromatographic profiles of the vitamin D sulfates in negative (underivatised compounds) and positive (derivatized with PTAD) ESI modes, as described in Section 2.

Table 1
Optimised mass spectrometry parameters used in MRM mode for all vitamin D analogues.

Compound	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	Fragmentor (*V*)	Collision energy (*V*)
D_2-S	475	96	242	32
D_2-S-d_3	477	96	242	32
D_3-S	463	96	232	36
D_3-S-d_3	465	96	232	36
25(OH)D_2-S	491	96	222	40
25(OH)D_2-S-d_3	493	96	222	40
25(OH)D_3-S	480	96	222	36
25(OH)D_3-S-d_3	482	96	222	36
D_2-S-PTAD	652	378	134	16
D_3-S-PTAD	640	378	124	12
25(OH)D_2-S-PTAD	650	378	154	12
25(OH)D_3-S-PTAD	639	378	144	12

possesses a potent antirachitic activity when administered in rats [11].

To our knowledge, the Vitamin D metabolite, 25-hydroxyvitamin D_2-sulfate (25(OH)D_2-S) has not yet been reported in biological fluids, and its physiological functions are not known. In fact, the vital role of D-S forms is still questioned and clear evidence for their biological function in humans has not yet been reported. A potential reason for this is the absence of