



# Combined collision-induced dissociation and photo-selected reaction monitoring mass spectrometry modes for simultaneous analysis of coagulation factors and estrogens

Quentin Enjalbert, Marion Girod, Jérémy Jeudy, Jordane Biarc, Romain Simon, Rodolphe Antoine, Philippe Dugourd, Jérôme Lemoine, Arnaud Salvador

## ► To cite this version:

Quentin Enjalbert, Marion Girod, Jérémy Jeudy, Jordane Biarc, Romain Simon, et al.. Combined collision-induced dissociation and photo-selected reaction monitoring mass spectrometry modes for simultaneous analysis of coagulation factors and estrogens. *Journal of Pharmaceutical Analysis*, 2014, 4 (3), pp.183-189. 10.1016/j.jpha.2013.09.004 . hal-01356364

**HAL Id: hal-01356364**

**<https://hal.science/hal-01356364>**

Submitted on 4 Sep 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



ORIGINAL ARTICLE

# Combined collision-induced dissociation and photo-selected reaction monitoring mass spectrometry modes for simultaneous analysis of coagulation factors and estrogens



Quentin Enjalbert<sup>a,b,c</sup>, Marion Girod<sup>a,c</sup>, Jérémy Jeudy<sup>a,c</sup>,  
Jordane Biarc<sup>a,c</sup>, Romain Simon<sup>a,c</sup>, Rodolphe Antoine<sup>a,b</sup>,  
Philippe Dugourd<sup>a,b</sup>, Jérôme Lemoine<sup>a,c</sup>, Arnaud Salvador<sup>a,c,\*</sup>

<sup>a</sup>Université Lyon 1, CNRS, Université de Lyon, 69622 Villeurbanne cedex, France

<sup>b</sup>Institut Lumière Matière, UMR5306, France

<sup>c</sup>Institut des Sciences Analytiques, UMR 5280, France

Received 28 March 2013; accepted 10 September 2013

Available online 17 September 2013

## KEYWORDS

Estrogen;  
Coagulation factor  
protein;  
Metabolite;  
Photo-dissociation  
fragmentation;  
SRM

**Abstract** Oral estrogens are directly associated with changes in plasma levels of coagulation proteins. Thus, the detection of any variation in protein concentrations due to estrogen contraceptives, by a simultaneous analysis of both coagulation proteins and estrogens, would be a very informative tool. In the present study, the merit of photo-selected reaction monitoring (SRM), a new analytical tool, was evaluated towards estrogens detection in plasma. Then, SRM and photo-SRM detection modes were combined for the simultaneous analysis of estrogen molecules together with heparin co-factor and factor XIIa, two proteins involved in the coagulation cascade. This study shows that photo-SRM could open new multiplexed analytical routes.

© 2013 Xi'an Jiaotong University. Production and hosting by Elsevier B.V.

Open access under [CC BY-NC-ND license](#).

\*Corresponding author at: Institut des Sciences Analytiques, UMR 5280, France. Tel.: +33 4 37 42 35 49; fax: +33 4 37 42 37 00.

E-mail address: [arnaud.salvador@univ-lyon1.fr](mailto:arnaud.salvador@univ-lyon1.fr) (A. Salvador).

Peer review under responsibility of Xi'an Jiaotong University.



Production and hosting by Elsevier

## 1. Introduction

Oral contraceptives were introduced in the late 1950s and became one of the most popular contraceptive tools. However, it is now well known that the use of hormonal contraceptives such as estrogens is associated with an increased risk of thromboembolic events [1–3]. Indeed, oral estrogens, and in particular ethinyl estradiol (EE2), are directly associated with changes in plasma levels of many coagulation proteins [4,5]. Hence, simultaneous

quantification of proteins involved in the coagulation process and level of circulating estrogen could be interesting for deciphering the relationship between estrogens and imbalance of coagulation homeostasis. The merits of liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) in selected reaction monitoring (SRM) mode have been widely illustrated for natural or synthetic estrogens analysis in plasma [6–8], and in urine [9] or in waste water [10–12]. The described methods usually involve estrogens derivatization with dansyl chloride in order to achieve the lowest limits of quantification (LOQ), typically in the pg/mL range, [6,12,13]. Another possibility consists of the use of 2D chromatography without derivatization [14]. Furthermore, most of these methods combine a liquid/liquid extraction (LLE), a solid-phase extraction (SPE) or a precipitation step to remove the bulk of plasma proteins [13,15,16]. However, such sample workflow does not appear suitable when protein levels are also informative. Recently, there have been reports of how protein assay associated with targeted mass spectrometry through the quantification of a proteotypic peptide is a promising alternative to immunotesting [17–22]. On the other hand, assay development for weakly concentrated proteotypic peptides is much more complex owing to sample complexity and dynamic concentration range of the whole proteome trypsin digest. Significant improvements of detection specificity have been recently obtained by introducing either an additional fragmentation step [23,24] or an accurate measurement of the fragment ions [25]. Another alternative strategy called photo-SRM [26,27] proposes the implementation of a laser photo-dissociation in a classical triple quadrupole in order to selectively fragment only chromophore-tagged compounds. In the present study, we combined for the first time a conventional collision fragmentation and a photo-dissociation fragmentation in a SRM mode for the simultaneous detection of estrogens and coagulation protein factors within a single chromatographic run.

## 2. Experimental

### 2.1. Reagents and chemicals

Acetonitrile (ACN), methanol (MeOH) and water (LC–MS grade) were obtained from Fisher Scientific (Strasbourg, France). Dithiothreitol (DTT), iodoacetamide (IAM), formic acid (FA) (LC–MS grade), trypsin (type IX-S from Porcine Pancreas), urea, ammonium bicarbonate (AMBIC), tris(2-carboxyethyl)phosphine (TCEP), sodium bicarbonate (NaHCO<sub>3</sub>), sodium hydroxide (NaOH) and dansyl chloride were purchased from Sigma-Aldrich (St Quentin-Fallavier, France). The pools of human plasma (men or menopause women) were

obtained from the Institut Pasteur (Lille, France). Internal standards estradiol-d2 (E2D2, purity = 97%) and ethinyl estradiol-d4 (EE2D4, purity = 97%) were obtained from CDN isotopes (Pointe-Claire, Canada).

### 2.2. Instrumentation

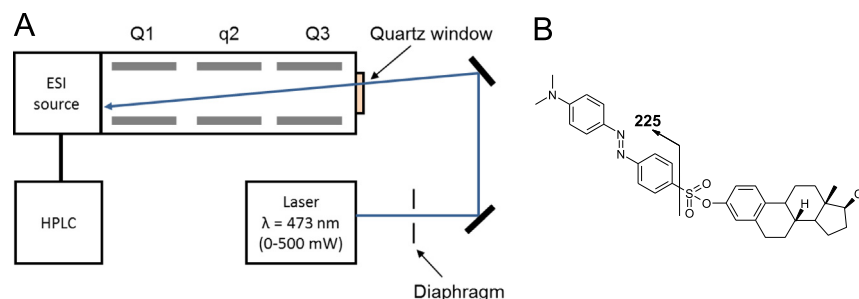
SRM and photo-SRM analyses were performed on a 4000 QTRAP<sup>®</sup> mass spectrometer (AB Sciex, Foster City, CA) equipped with a Turbo V<sup>™</sup> ion source coupled to an Agilent 1290 series high pressure liquid chromatography (Agilent Technologies, Waldbronn, Germany). A schematic of the photo-SRM set-up is given in Fig. 1A [27]. A quartz window was fitted on the rear of the MS instrument chamber to allow the introduction of a laser beam. The laser was a 473 nm continuous wavelength laser (cw) (ACAL BFI, Evry, France). The laser output power of the laser was 500 mW with a beam diameter of 0.6 mm (divergence 1.2 mrad). The laser beam passed through a single diaphragm and is injected into the MS instrument using two mirrors. To avoid fragmentation in Q1 and Q3, the laser beam was slightly off-axis ( $\sim 0.2^\circ$ ).

### 2.3. HPLC operating conditions

The HPLC separation was carried out on an Xselect<sup>™</sup> C<sub>18</sub> column (100 mm  $\times$  2.1 mm, 3.5  $\mu$ m) from Waters (Milford, MA, USA). The mobile phase consisted of water containing 0.1% (v/v) formic acid as eluent A and ACN containing 0.1% (v/v) formic acid as eluent B. Twenty microlitre of each sample were injected for both analytical methods. For analysis of estrogens alone, elution was performed at a flow rate of 300  $\mu$ L/min for 5 min with an isocratic elution of 5% of eluent A and 95% of eluent B. The simultaneous analysis of peptides and estrogens were performed during 16 min with an elution including a 2 min isocratic period with 95% of eluent A, followed by a 6 min linear gradient from 95% to 70% of eluent A and a column washing at 100% of eluent B for 4 min. The gradient returned to the initial conditions for 4 min, before the next injection.

### 2.4. Mass spectrometry operating conditions

Ionization was achieved using electrospray in positive ionization mode with an ion spray voltage of 5500 V. The following conditions were found to be the optimal conditions for the analysis of E2 and EE2 in SRM and photo-SRM methods. The curtain gas flow (nitrogen), the ion source gas 1 and 2 (air) were respectively



**Fig. 1** (A) Schematic of the experimental set-up. (B) Chemical structure of E2 estradiol coupled with dansyl chloride, main fragment at 225  $m/z$  is shown.

set at 15, 50 and 20 arbitrary units. The Turbo ion spray source was operating at 450 °C. Q1 and Q3 quadrupole resolutions were adjusted to  $0.7 \pm 0.1$  amu. Collision energy (CE) was set to 40 eV for CID experiments and to 5 eV for photo-SRM (to avoid CID).

For complex analyses, a mass spectrometry method with two different periods, over the analysis time, was developed. The first period from 0 to 8 min was developed to analyze common proteotypic peptides and the following experimental conditions were optimized for peptide quantification using MRM Pilot software™ (AB Sciex, Foster City, CA, USA). Ionization was achieved using electrospray in positive ionization mode with an ion spray voltage of 5500 V. The curtain gas flow (nitrogen), the ion source gas 1 and 2 (air) were respectively set at 50, 50 and 20 units. The Turbo ion spray source was operating at 450 °C. Collision energies and SRM transitions are shown in Table S1 (Electronic supplementary material). Q1 and Q3 quadrupole resolutions were adjusted to  $0.7 \pm 0.1$  amu. The second period, from 8 to 16 min was developed to analyze estrogens. The source and mass spectrometry conditions used for SRM and photo-SRM methods were the same as those for the rapid analysis.

### 2.5. Sample preparation

Prior to any sample preparation, a concentration range of 0, 200, 500, 2000, 5000, 10,000 and 20,000 pg/mL of estrogen internal standards were prepared in a solution of sodium bicarbonate buffer (100 mM, pH adjusted to 10 with NaOH).

For rapid estrogens analyses, 10 µL of each estrogen internal standard solution were spiked in 90 µL of plasma to obtain a concentration range of 0, 20, 50, 200, 500 and 2000 pg/mL. Nine hundred microlitre of ACN were then added to each sample to precipitate proteins. Then, samples were centrifuged (10 min, 15,000 rpm, room temperature (RT)) and 900 µL of the upper layer were collected and concentrated to dryness under a stream of nitrogen. The residue of each tube was redissolved in 70 µL of sodium bicarbonate buffer (100 mM, pH adjusted to 10 with NaOH) followed by vortex-mixing for 1 min. To each sample, 30 µL of dabsyl chloride solution (1 mg/mL in acetone) were added followed by vortex-mixing for 1 min. Samples were placed in a 60 °C incubator for 10 min, then cooled and stored at 4 °C before analysis. Triplicates of each standard were realized.

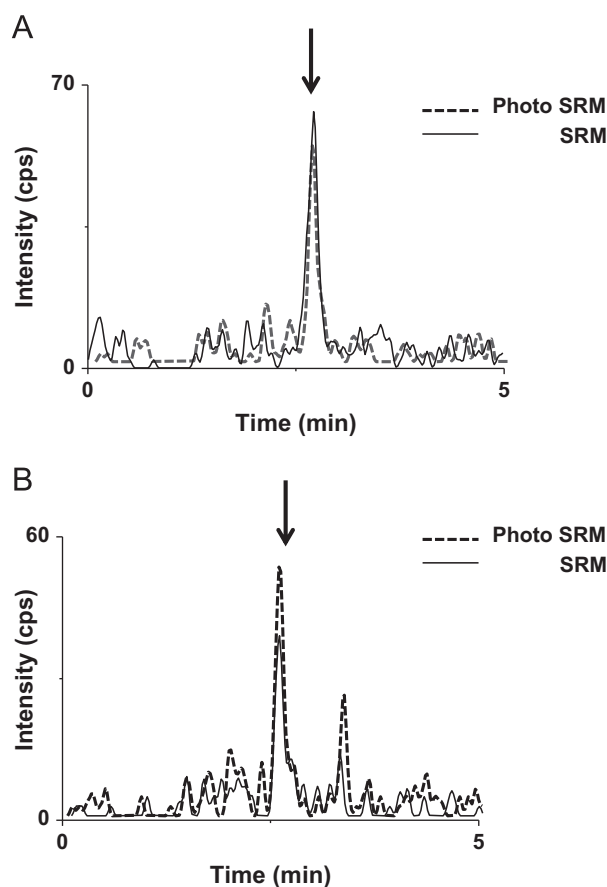
For complex analysis, 10 µL of each solution of estrogen internal standards were spiked in 90 µL of plasma to obtain a range of concentration of 0, 20, 50, 200, 500, 1000 and 2000 pg/mL. For peptide alkylation and estrogen derivatization, samples were denatured with 400 µL of 8 M urea (pH=10). 55 µL of 150 mM dithiothreitol and 100 µL of dabsyl chloride solution (1 mg/mL in acetone) were added to the samples before warming-up at 60 °C for 40 min. Samples were cooled to RT and alkylated with 170 µL of 150 mM iodoacetamide at RT in the dark for 40 min. To reduce the urea concentration, the samples were diluted 5-fold with AMBIC (50 mM) prior to overnight digestion at 37 °C with trypsin using a 1:30 (w/w) enzyme to substrate ratio. All samples were desalted and concentrated using Oasis™ HLB 3 cm<sup>3</sup> (60 mg) reversed phase cartridges (Waters, Milford, MA, USA). Before loading the tryptic digest onto the Oasis cartridges, all cartridges were conditioned with 1 mL of MeOH and 1 mL of water containing 0.5% FA. After the loading, all cartridges were washed with 1 mL of MeOH/water (5/95, v/v+0.5% FA) and eluted with 2 mL of ACN containing 0.5% FA. The samples were evaporated to dryness under a stream of nitrogen. The residue of

each tube was redissolved in 100 µL of sodium bicarbonate buffer (100 mM, pH adjusted to 10 with NaOH) followed by vortex-mixing for 1 min. Samples were cooled and stored at 4 °C until analysis.

## 3. Results and discussion

### 3.1. Comparison of SRM and photo-SRM for single estrogen analysis

Tandem mass spectrometry analyses on a triple quadrupole analyzer are based on the collision induced dissociation (CID) process with a detection specificity brought through two mass selections in Q1 and Q3 (called SRM transition). Usually, CID-SRM selectivity is sufficient for quantification of small molecules. However, in complex matrices such as plasma or serum after trypsin digestion, co-eluted interferences with the same SRM transition can be detected. In order to add a new selectivity step, the CID process has been substituted with laser induced dissociation (LID), allowing measurement by LID-SRM or photo-SRM. Indeed, the CID process is a non-discriminating fragmentation mode where all ions selected in Q1 are fragmented while LID can

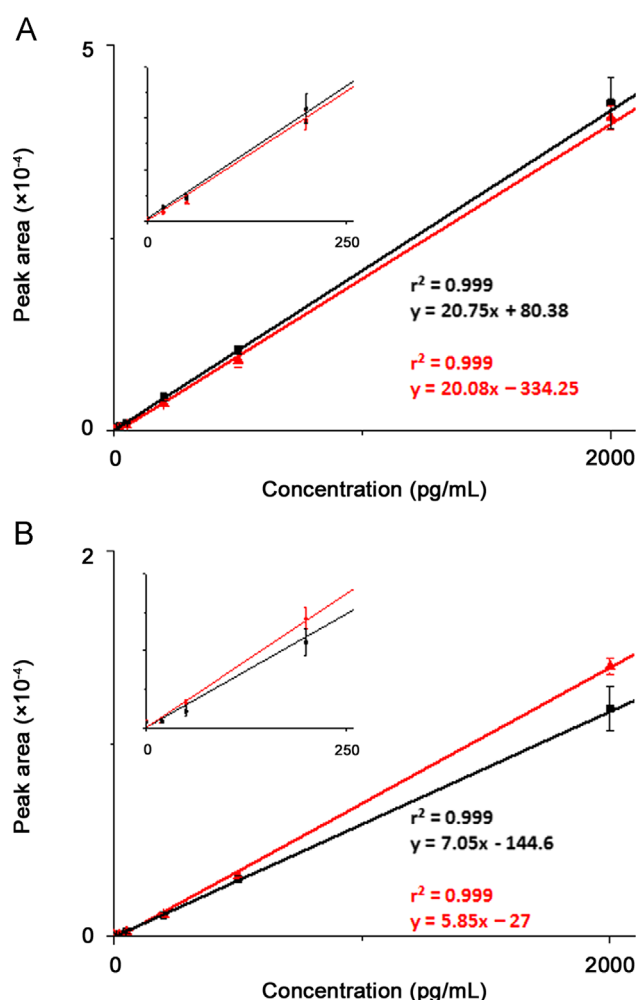


**Fig. 2** LC-MS/MS chromatograms tracking down either E2D2 (SRM transition 562.5/225.0, spiked at 20 pg/mL) in human plasma obtained following photo-SRM and SRM analyses (A), or EE2D4 (SRM transition 588.5/225.0, spiked at 50 pg/mL) in human plasma obtained following photo-SRM and SRM analyses (B). The arrows show target molecules.

only be used after the molecules have absorbed photons of the chosen wavelength. The majority of biomolecules available in plasma or serum do not absorb in the visible wavelength range, so the use of a laser emitting at 473 nm photo-fragments exclusively molecules absorbing at this wavelength. Thus, in order to bring the correct optical properties to estrogens, a chromophore-derivatization is required. The dansyl chloride chromophore, usually used for estrogens derivatization, does not absorb at 473 nm, so it has been substituted with the dabsyl chloride chromophore [28]. Gas phase optical spectra, recorded on a linear ion trap coupled with an optical parametric oscillator laser [29–31], showed a high absorption at 473 nm with a  $\lambda_{\text{max}} = 490$  nm (Fig. S1 in electronic supplementary material).

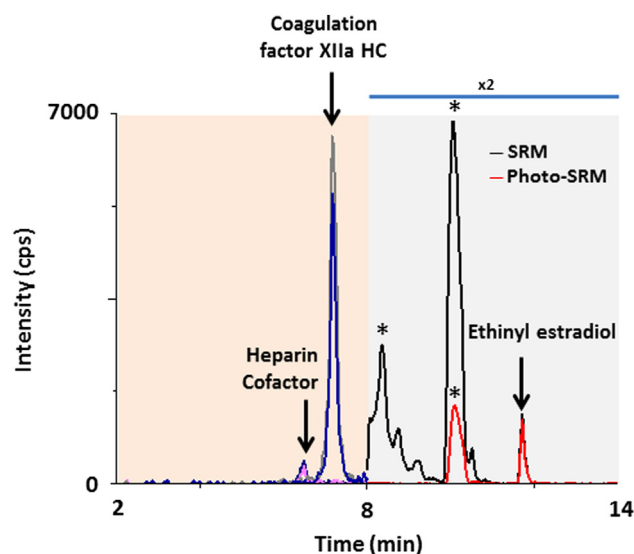
Prior to simultaneous analysis of estrogens and proteins in the same run, the benefit of photo-SRM has been evaluated and compared with the basic CID-SRM method for the quantification of estrogens in plasma samples. Thus, a wide concentration range of internal standards E2D2 and EE2D4 (0–2000 pg/mL) has been prepared in senior women plasma. LID and CID spectra of the derivatized estrogens resulted in an intense fragment ion at  $m/z$  225, corresponding to a fragmentation within the chromophore (see Fig. 1B). The couple precursor ion/fragment ion ([derivatized estrogen+H]<sup>+</sup>/225) was used in both SRM methods for the

analysis of derivatized estradiol compound E2D2 (Fig. 2B) and derivatized ethinyl estradiol compound EE2D4 (Fig. 2B). As shown in Fig. 2, following plasma precipitation, no major interference was detected either in CID-SRM (solid line) or in photo-SRM channel (dotted line). For estradiol, the signal intensities were similar in both methods while the photo-SRM signal detected for ethinyl estradiol was slightly improved in comparison to the SRM. These results show that photo-SRM could be an alternative to CID-SRM for quantitation of estrogens, especially in very complex matrix as it will be illustrated in Section 3.2. In addition, both estrogens were either spiked before or after the protein depletion by precipitation and the signal intensities were compared to detect any loss of target molecules due to the precipitation. No major signal difference was observed, indicating that estrogen quantification was not biased by protein precipitation process. Calibrations curves of SRM and photo-SRM transitions obtained for estradiol and ethinyl estradiol compounds are shown in Fig. 3. The experiments have been performed in triplicate over the full experimental sample workflow (i.e., chromophore tagging, sample precipitation). The calculated linearity shows that robustness and repeatability can be validated for the whole analytical process. Back calculated accuracies (accuracies are expressed as percent difference) also show good robustness and repeatability with



**Fig. 3** Calibration curves of SRM (in black ■) and photo-SRM (in red ▲) transition obtained for the analyses of E2D2 (A) and EE2D4 (B). Insets show low concentrations (0–250 pg/mL).





**Fig. 4** LC-MS/MS chromatograms tracking down coagulation proteins and estrogens in two different periods. From 0 to 8 min, 2 SRM transitions for heparin cofactor (RT=6.5 min) and 2 SRM transitions for coagulation factor XIIa HC (RT=7.1 min). From 8 to 16 min, ethinyl estradiol either in SRM mode (black line) or in photo-SRM mode (red line) SRM transition 588.5/225.0, spiked at 500 pg/mL, RT=11.9 min in human plasma. All \* represent interferences detected in SRM and photo-SRM modes.

values lower than 15% over the whole concentration range. The two methods are thus comparable for the quantification and detection of target molecules. However, previous publications demonstrated that photo-SRM is more interesting in the case of very complex matrix, such as digested plasma, where peptides and small molecules are still present [26].

### 3.2. Simultaneous analysis

Once the proof of concept for photo-SRM estrogen analysis has been done, a detection of labeled estrogens and coagulation proteins has been performed to measure simultaneously both biomolecules from the same sample during the same run. In order to avoid any variation of coagulation protein concentrations, a pool of menopausal female plasma has been chosen. As all target molecules have to be retained in the same fraction, the precipitation process has been replaced by a classic protein quantification protocol digesting proteins into peptides as described in Section 2. Traditionally, estrogens derivatization is made in sodium bicarbonate buffer. However, urea (8 M, pH=10), which also acts as denaturant before protein digestion, was directly used as derivatization buffer. The LC method for simultaneous analysis was sequenced in two different periods of 8 min each. The first period consisted of a CID-SRM analysis of proteotypic peptides of two coagulation proteins (Coagulation factor XIIa HC and Heparin Cofactor). SRM transitions values of proteotypic peptides are based on commonly used values reported by Hortin et al. [32]. The two targeted peptides were detected in CID-SRM on a time scale shorter than 8 min (less than 30% of eluent B) as they are less hydrophobic than the derivatized estrogens. Fig. S2 (in electronic supplementary material) shows MS/MS experiments performed to identify the two proteotypic peptides while Table S1 (in electronic supplementary material) shows SRM transitions collision energies used for CID fragmentation, chromatographic peak heights, chromatographic peak areas and retention times recorded for the two proteotypic peptides. The second period of the LC method

consisted in the analysis of estrogens compounds either in SRM or photo-SRM.

Fig. 4 shows the whole reconstructed chromatogram tracking down, from 0 to 8 min, the two endogenous coagulation proteins (Coagulation factor XIIa HC and Heparin Cofactor) by SRM and from 8 to 16 min, the ethinyl estradiol compound recorded in SRM or photo-SRM. Major interferences were detected between 8 and 12 min during the SRM method, especially through the estrogen elution. This is due to the fact that, compared with the first experiment measuring only estrogens, proteins were not precipitated during the sample preparation and are still present. On the other hand, almost no interfering peaks are detected during the photo-SRM mode. The comparison of both methods clearly shows the drastic simplification of chromatograms obtained with photo-SRM vs. SRM, proving that classical SRM detection specificity is not high enough for a simultaneous detection. As a consequence, the increased specificity of the photo-dissociation process could extend the response linearity in case of co-eluting compounds from the complex matrix. In this preliminary results, analytical validation on proteins was not performed, however multiplexed quantitative proteomics have been well described and are commonly used [33,34]. Thus, further experiments involving several coagulation proteins and estrogens should be performed in future work.

## 4. Conclusion

Here is presented a new application of the photo-SRM mode for the detection and quantification of estrogens in complex matrices. For single estrogen quantification, photo-SRM method is as sensitive as classic SRM mode without any loss of specificity or sensitivity. Moreover, in a case of a simultaneous analysis of small molecules and proteins, in very complex matrices, CID-SRM combined to photo-SRM could improve the detection specificity of estrogens as a result of a more specific fragmentation step and by

the increased hydrophobicity of chromophore derivatized compounds. These results are very encouraging and in perspective, the mass spectrometer sensitivity could be increased by implementing the photo-SRM method in a last generation triple quadrupole. Finally, the single use of photo-SRM method could be applied for the simultaneous detection of peptides and small molecules. In that case, we should either use the same chromophore (such as dabsyl chloride) for small molecules and peptides to detect all biomolecules with a phenol function or use two different chromophores to tag two different chemical functions. Thus, new analytical routes could be investigated as “metabo-proteomics” analysis.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.jpha.2013.09.004>.

## References

- [1] J.P. Vandenbroucke, J. Rosing, K.W.M. Bloemenkamp, et al., Oral contraceptives and the risk of venous thrombosis, *N. Engl. J. Med.* 344 (2001) 1527–1535.
- [2] G. Tans, J. Curvers, S. Middeldorp, et al., A randomized cross-over study on the effects of levonorgestrel- and desogestrel-containing oral contraceptives on the anticoagulant pathways, *Thromb. Haemostasis* 84 (2000) 15–21.
- [3] F.R. Rosendaal, Venous thrombosis: the role of genes, environment, and behavior, *Hematology/the Education Program of the American Society of Hematology, American Society hematology, Education Program, ASH Education Book*, 2005, pp. 11–12. <http://dx.doi.org/10.1182/asheducation-2005.1.1> (<http://asheducationbook.hematologylibrary.org/content/2005/1/1.full#>).
- [4] A.C.A. Cleuren, I.K. Van der Linden, Y.P. De Visser, et al., 17 alpha-Ethinylestradiol rapidly alters transcript levels of murine coagulation genes via estrogen receptor alpha, *J. Thromb. Haemost.* 8 (2010) 1838–1846.
- [5] J.L. Ockrim, E.N. Lalani, A.K. Kakkar, et al., Transdermal estradiol therapy for prostate cancer reduces thrombophilic activation and protects against thromboembolism, *J. Urol.* 174 (2005) 527–532.
- [6] K. Rangiah, S.J. Shah, A. Vachani, et al., Liquid chromatography/mass spectrometry of pre-ionized Girard P derivatives for quantifying estrone and its metabolites in serum from postmenopausal women, *Rapid Commun. Mass Spectrom.* 25 (2011) 1297–1307.
- [7] R.E. Nelson, S.K. Grebe, D.J. O’Kane, et al., Liquid chromatography-tandem mass spectrometry assay for simultaneous measurement of estradiol and estrone in human plasma, *Clin. Chem.* 50 (2004) 373–384.
- [8] R.W. Giese, Measurement of endogenous estrogens: analytical challenges and recent advances, *J. Chromatogr. A* 1000 (2003) 401–412.
- [9] C.H. Huang, D.L. Sedlak, Analysis of estrogenic hormones in municipal wastewater effluent and surface water using enzyme-linked immunosorbent assay and gas chromatography/tandem mass spectrometry, *Environ. Toxicol. Chem.* 20 (2001) 133–139.
- [10] T. Vega-Morales, Z. Sosa-Ferrera, J.J. Santana-Rodriguez, Determination of various estradiol mimicking-compounds in sewage sludge by the combination of microwave-assisted extraction and LC–MS/MS, *Talanta* 85 (2011) 1825–1834.
- [11] V. Gabet-Giraud, C. Miegé, B. Herbreteau, et al., Development and validation of an analytical method by LC–MS/MS for the quantification of estrogens in sewage sludge, *Anal. Bioanal. Chem.* 396 (2010) 1841–1851.
- [12] A. Salvador, C. Moreton, A. Píram, et al., On-line solid-phase extraction with on-support derivatization for high-sensitivity liquid chromatography tandem mass spectrometry of estrogens in influent/effluent of wastewater treatment plants, *J. Chromatogr. A* 1145 (2007) 102–109.
- [13] M.R. Anari, R. Bakhtiar, B. Zhu, et al., Derivatization of ethinylestradiol with dansyl chloride to enhance electrospray ionization: application in trace analysis of ethinylestradiol in rhesus monkey plasma, *Anal. Chem.* 74 (2002) 4136–4144.
- [14] T. Fiers, B. Casetta, B. Bernaert, et al., Development of a highly sensitive method for the quantification of estrone and estradiol in serum by liquid chromatography tandem mass spectrometry without derivatization, *J. Chromatogr. B* 893 (2012) 57–62.
- [15] C. Fernandez, H.M. Santos, C. Ruiz-Romero, et al., A comparison of depletion versus equalization for reducing high-abundance proteins in human serum, *Electrophoresis* 32 (2011) 2966–2974.
- [16] S.E. Warder, L.A. Tucker, T.J. Streltzer, et al., Reducing agent-mediated precipitation of high-abundance plasma proteins, *Anal. Biochem.* 387 (2009) 184–193.
- [17] H. Keshishian, T. Addona, M. Burgess, et al., Quantification of cardiovascular biomarkers in patient plasma by targeted mass spectrometry and stable isotope dilution, *Mol. Cell. Proteomics* 8 (2009) 2339–2349.
- [18] D.S. Kirkpatrick, S.A. Gerber, S.P. Gygi, The absolute quantification strategy: a general procedure for the quantification of proteins and post-translational modifications, *Methods* 35 (2005) 265–273.
- [19] R. Simon, M. Girod, C. Fonbonne, et al., Total ApoE and ApoE4 isoform assays in an Alzheimer’s disease case-control study by targeted mass spectrometry ( $n=669$ ): a pilot assay for methionine-containing proteotypic peptides, *Mol. Cell. Proteomics* 11 (2012) 1389–1403.
- [20] L. Anderson, C.L. Hunter, Quantitative mass spectrometric multiple reaction monitoring assays for major plasma proteins, *Mol. Cell. Proteomics* 5 (2006) 573–588.
- [21] N.L. Anderson, N.G. Anderson, L.R. Haines, et al., Mass spectrometric quantitation of peptides and proteins using stable isotope standards and capture by anti-peptide antibodies (SISCAPA), *J. Proteome Res* 3 (2004) 235–244.
- [22] S. Gallien, E. Duriez, B. Domon, Selected reaction monitoring applied to proteomics, *J. Mass Spectrom.* 46 (2011) 298–312.
- [23] J. Lemoine, T. Fortin, A. Salvador, et al., The current status of clinical proteomics and the use of MRM and MRM3 for biomarker validation, *Expert Rev. Mol. Diagn.* 12 (2012) 333–342.
- [24] T. Fortin, A. Salvador, J.P. Charrier, et al., Multiple reaction monitoring cubed for protein quantification at the low nanogram/milliliter level in nondepleted human serum, *Anal. Chem.* 81 (2009) 9343–9352.
- [25] A.C. Peterson, J.D. Russell, D.J. Bailey, et al., Parallel reaction monitoring for high resolution and high mass accuracy quantitative, targeted proteomics, *Mol. Cell. Proteomics* 11 (2012) 1475–1488.
- [26] Q. Enjalbert, M. Girod, R. Simon, et al., Improved detection specificity for plasma proteins by targeting cysteine-containing peptides with photo-SRM, *Anal. Bioanal. Chem.* 405 (2013) 2321–2331.
- [27] Q. Enjalbert, R. Simon, A. Salvador, et al., Photo-SRM: laser-induced dissociation improves detection selectivity of selected reaction monitoring mode, *Rapid Commun. Mass Spec.* 25 (2011) 3375–3381.
- [28] E. De Mey, G. Drabik-Markiewicz, H. De Maere, et al., Dabsyl derivatization as an alternative for dansylation in the detection of biogenic amines in fermented meat products by reversed phase high performance liquid chromatography, *Food Chem.* 130 (2012) 1017–1023.
- [29] R. Antoine, P. Dugourd, Visible and ultraviolet spectroscopy of gas phase protein ions, *Phys. Chem. Chem. Phys.* 13 (2011) 16494–16509.
- [30] V. Larrailet, R. Antoine, P. Dugourd, et al., Activated-electron photodetachment dissociation for the structural characterization of protein polyanions, *Anal. Chem.* 81 (2009) 8410–8416.
- [31] A. Racaud, R. Antoine, L. Joly, et al., Wavelength-tunable ultraviolet photodissociation (UVPD) of Heparin-derived disaccharides in a linear ion trap, *J. Am. Soc. Mass Spec.* 20 (2009) 1645–1651.

- [32] G.L. Hortin, D. Sviridov, N.L. Anderson, High-abundance polypeptides of the human plasma proteome comprising the top 4 logs of polypeptide abundance, *Clin. Chem.* 54 (2008) 1608–1616.
- [33] H. Keshishian, T. Addona, M. Burgess, et al., Quantitative, multiplexed assays for low abundance proteins in plasma by targeted mass spectrometry and stable isotope dilution, *Mol. Cell. Proteomics* 6 (2007) 2212–2229.
- [34] P.J. Boersema, R. Raijmakers, S. Lemeer, et al., Multiplex peptide stable isotope dimethyl labeling for quantitative proteomics, *Nat. Protoc.* 4 (2009) 484–494.