

Understanding the Response of the Molecule to Electrospray Ionization (ESI) by Selected Reaction Monitoring (SRM) Tandem Mass Spectrometry: Defining Molecular ESI Index (MESII)

R Guleria¹, S Pasha², G Makharia¹ and V Sabareesh^{2*}¹Department of Gastroenterology and Human Nutrition Unit, All India Institute of Medical Sciences, Ansari Nagar, New Delhi - 110029, India²Proteomics and Structural Biology Unit, Council of Scientific and Industrial Research - Institute of Genomics and Integrative Biology, Mall Road, Delhi - 110007, India**Abstract**

In an attempt to understand the response of the molecule to electrospray ionization (ESI), a new parameter called 'molecular electrospray ionization index (MESII)' is defined. Selected reaction monitoring (SRM) data acquired on an ESI tandem quadrupole mass spectrometer is utilized for calculating MESII, which we denote as ϵ_{SRM} :

$$\epsilon_{\text{SRM}} = -\log(\text{SRM intensity/Population of ions or molecules in-solution}).$$

Population of ions or molecules in-solution is estimated using molecular mass of the respective compound and Avogadro's constant. Simvastatin acid (SVA), lovastatin (LV) and simvastatin (SV) are chosen as model compounds. SRM experiments in positive ion mode were performed on singly protonated ions ($[M+H]^+$) of SVA, LV and SV. In negative ion mode, only SVA was investigated by SRM, using singly deprotonated ion ($[M-H]^-$) as precursor ion. Thus estimated MESII values in positive ion mode are: ϵ_{SRM}^+ (SVA)=7.4288, ϵ_{SRM}^+ (LV)=7.4541 and ϵ_{SRM}^+ (SV)=8.6833 and in negative ion mode, ϵ_{SRM}^- (SVA)=7.2253. This newly defined index not only gives an idea about ionization potential (i.e., degree of ionization), but can also be an indicator of limit of detection (LOD) of an analyte. When utilizing SRM data recorded from different type of instruments for a compound, the variations that may arise in MESII values could help in understanding the influence of different instrument configurations/methods on the degree of ionization of that compound. Matrix effects on the extent of analyte's ionization too can be understood from the differences in the MESII values. Further, it may be possible to utilize MESII for quantitation as well.

Keywords: Mass spectrometry; Electrospray ionization; Tandem mass spectrometry; Collision induced dissociation; Selected reaction monitoring; Ionization potential

Introduction

The process of generating ions in gas phase is the foremost as well as a critical step in mass spectrometry (MS). In many cases, the molecular ions would be already present in solution phase and the ionization mode of MS would then transfer the ions from liquid phase to gas phase. Also, there are several examples, wherein the processes involved in an ionization mode would be converting the neutrals available in solution/solid to gas phase molecular ions, which are then characterized based on their mass-to-charge ratios (m/z). Subject to the conditions (physical) followed during the process of (gas phase) ionization and depending on the chemical property, different molecules respond or ionize to varied extents. For example, a compound which may not ionize properly by atmospheric pressure chemical ionization (APCI) could ionize very well through electrospray ionization (ESI) and vice-versa. Further, the yield of ions generated in gas phase through an ionization mode, could differ from one analyte molecule to another, though the concentration of both the analytes can be same. Moreover, certain molecules/molecular ions dissociate during the ionization process, which is called in-source fragmentation and this is again dependent on the analyte molecule and mode of ionization.

Thus, in order to understand the response of the analyte molecule to an ionization process, a suitable yardstick or parameter is required. For example, 'proton affinity', 'gas phase basicity' and 'gas phase acidity' values that are typically estimated by Cooks' kinetic method are considered as indicators of degree of ionization of an analyte [1]. Cooks' kinetic method involves a standard reference compound (SRC), wherein proton bound dimer formed by the analyte under study and the SRC, would be subjected to collision induced dissociation (CID) and the abundances of resulting product ions are used to estimate proton affinity or gas phase acidity of the analyte of interest [1-3]. In this study,

a new parameter called 'molecular ESI index (MESII)' is defined, which also can be useful to comprehend, how different analytes respond to ESI. MESII of an analyte is calculated using the intensity values of selected reaction monitoring (SRM) experiment of an adduct ion, e.g., $[M+H]^+$ and the molar concentration of that analyte.

Simvastatin acid (SVA), lovastatin (LV) and simvastatin (SV) were chosen as models (Figure 1) in this investigation to understand their response to ESI by SRM through the new definition of MESII. Although ESI coupled to SRM has been used to characterize LV and SV, especially in clinically relevant contexts [4-8], there are no reports on determination of proton affinity and gas phase acidity of these compounds. LV and SV are very widely used cholesterol lowering pro-drugs; SVA, the β -hydroxy acid form of SV, is a potent competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, an enzyme involved in cholesterol biosynthesis [9].

Experimental

Working solutions of SVA, LV and SV at concentrations 1, 5, 10, 25, 50, 75, 100, 250 and 500 $\mu\text{g mL}^{-1}$ were prepared by successive dilution

*Corresponding author: V. Sabareesh, Advanced Centre for Bio Separation Technology (CBST), Vellore Institute of Technology University, Vellore, Tamil Nadu - 632014, India, Tel: +91-416 2202722, E-mail: sabareesh6@gmail.com / v.sabareesh@vit.ac.in.

Received August 20, 2015; Accepted September 07, 2015; Published September 14, 2015

Citation: Guleria R, Pasha S, Makharia G, Sabareesh V (2015) Understanding the Response of the Molecule to Electrospray Ionization (ESI) by Selected Reaction Monitoring (SRM) Tandem Mass Spectrometry: Defining Molecular ESI Index (MESII). Mass Spectrom Purif Tech 1: 104. doi:10.4172/2469-9861.1000104

Copyright: © 2015 Guleria R, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

of stock solutions using acetonitrile: water (60:40; v/v). The samples were introduced into the mass spectrometer through Acquity UPLC (Waters) following isocratic elution on Hypersep Gold C18 column (2.1 × 50 mm, 3 μm particle size; Thermo Scientific) for a total run time of 5 minutes employing H₂O (MilliQ, solvent A) and acetonitrile (ACN, solvent B) as mobile phase with composition of ACN:H₂O, 60:40, (v/v). Flow rate was set at 0.2 mL min⁻¹. For each run, 10 μL of the sample was injected onto the column from the autosampler maintained at 18°C. The SRM data were acquired on a tandem quadrupole mass spectrometer (Quattro Micro, Waters) equipped with ESI source; source and analyzer parameters of ESI are given in Table S1 (Supplementary Material). Through daughter scan experiments, collision energies for different SRM transitions were optimized (Table S2). Full scan and daughter scan (CID) mass spectra are shown in Figures S1 and S2, respectively. Figure S3 shows representative SRM chromatograms from 500 pg μL⁻¹ concentration of SVA, LV and SV.

Results

In positive ion polarity of ESI, singly protonated ions ([M+H]⁺) of SVA (*m/z* 437), LV (*m/z* 405) and SV (*m/z* 419) were detected (Figure S1). In negative ion polarity, only SVA yielded deprotonated ion ([M-H]⁻; *m/z* 435) (Figure S1), which can be attributed to the presence of a carboxylic functional group in SVA and therefore, SVA was subjected to both positive and negative ion SRM (Figure S3). SRM intensity values observed from different concentrations of SVA, LV and SV in positive and negative ion polarities are shown in Table S3. All data were acquired in triplicates. In SRM data, signal-to-noise (S/N) ratios were found to be in the range 6 - 10 for all concentrations; except in the case of 1 and 5 pg μL⁻¹ of SV, which did not yield significant signal intensities. For SVA, S/N ratios were found to be (in the range 7 - 10) higher than both LV and SV. LV showed better S/N ratios than SV for all concentrations.

SRM of SVA in positive and negative ion polarities

SRM intensities of SVA in negative ion mode are almost twice that of SVA in positive ion mode (Figure 2a, Table S3). This implies that the carboxyl group of SVA is a better proton donor than proton acceptor and therefore, SVA ionizes far better in negative ion mode of ESI than positive ion polarity. The SRM of SVA in negative ion polarity comprises of only one precursor ion → product ion transition (*m/z* 435 → *m/z* 319), as MS/MS of [M-H]⁻ of SVA (precursor ion *m/z* 435) consisted of only one fragment ion, *m/z* 319 (Figure S2a); there seems to be no other dissociation pathways for this anionic precursor *m/z* 435 [10]. This suggests that almost the entire population of parent ions (*m/z* 435) follows only one fragmentation pathway, yielding only a daughter ion at *m/z* 319 (Table S2) at an optimum CE and therefore, the transition *m/z* 435 → *m/z* 319 (in negative ion ESI) could provide a higher measure of the electrosprayed ions as compared to the transitions (*m/z* 437 → *m/z* 303 and *m/z* 437 → *m/z* 285) in positive ion ESI.

SRM of LV in positive ion polarity

SRM of LV in positive ion polarity comprises of two precursor ion → product ion transitions, which are *m/z* 405 → *m/z* 285 and *m/z* 405 → 199 (Table S2). The intensity values for LV are 12 to 20 fold higher than SV in positive ion polarity (Figure 2b, Table S3). In fact, for low concentrations (1 pg μL⁻¹ and 5 pg μL⁻¹) of LV, significant signals (having S/N ratios 7 and 9, respectively) were obtained in SRM chromatograms, whereas no signals yielded from SV at these concentrations. Such variations can be attributed to the molecular structures, since the ionization mode and other relevant (physical)

parameters were the same for data acquisitions of both LV and SV. SV differs from LV in having an extra methyl group (Figure 1) and perhaps the extra methyl in SV offers steric hindrance for the formation of protonated adduct ions (i.e., [M+H]⁺) of SV. This might be the reason for poorer ionization of SV than LV and hence significantly less SRM intensity values are observed for SV than LV. In contrast, SRM intensities of LV are very close to SVA (Figure 2c, Table S3). This can be ascribed to the presence of the free carboxyl group of SVA, which might aid in overriding the effect of steric impediment due to the extra methyl group in SVA; whereas the lactone ring in LV may not be a good proton acceptor as compared to β-hydroxy carboxylic function of SVA.

SRM of SV in positive ion polarity

For every concentration, the intensity of SVA in positive ion polarity is about 12 to 22 fold higher than SV (Figure 2d, Table S3). These intensity values are sum of the intensities from each of the two SRM transitions corresponding to [M+H]⁺ of SV (*m/z* 419 → 285 and *m/z* 419 → 199) and SVA (*m/z* 437 → 303 and *m/z* 437 → 285). For lower concentrations of 1 pg μL⁻¹ and 5 pg μL⁻¹, significantly better intensity values were noted for SVA than SV. Such a drastic variation in the intensity observed for same concentrations of SV and SVA, under same ionization conditions, can again be attributed to the molecular structures of SV and SVA (Figure 1); the key difference is that SV possesses lactone group, whereas SVA contains β-hydroxy carboxylic acid moiety. The observed trend indicates that binding of proton to the β-hydroxy carboxylic group of SVA is better than to the lactone group of SV, thereby producing higher abundance of protonated adduct

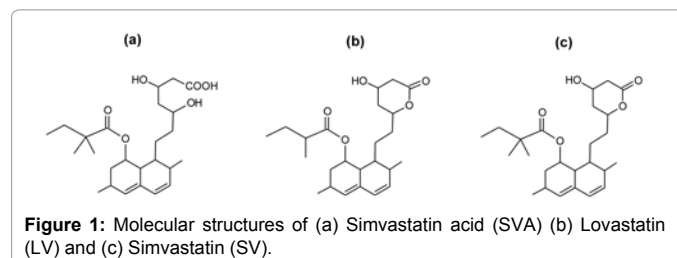


Figure 1: Molecular structures of (a) Simvastatin acid (SVA) (b) Lovastatin (LV) and (c) Simvastatin (SV).

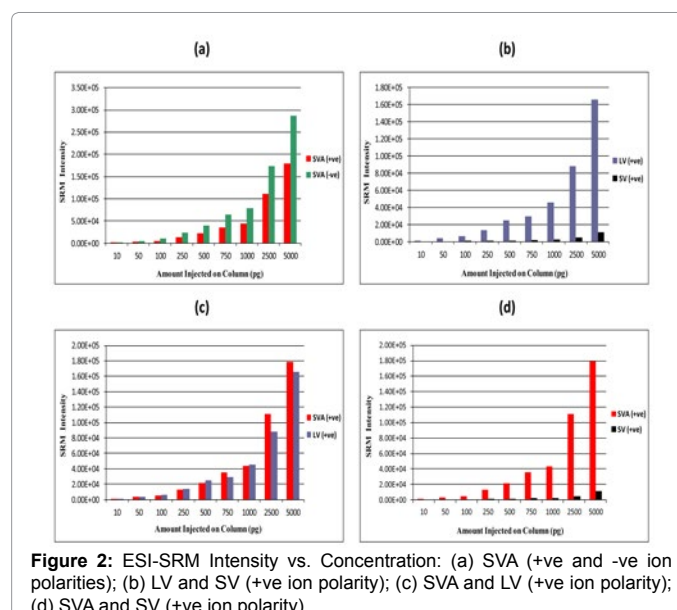


Figure 2: ESI-SRM Intensity vs. Concentration: (a) SVA (+ve and -ve ion polarities); (b) LV and SV (+ve ion polarity); (c) SVA and LV (+ve ion polarity); (d) SVA and SV (+ve ion polarity).

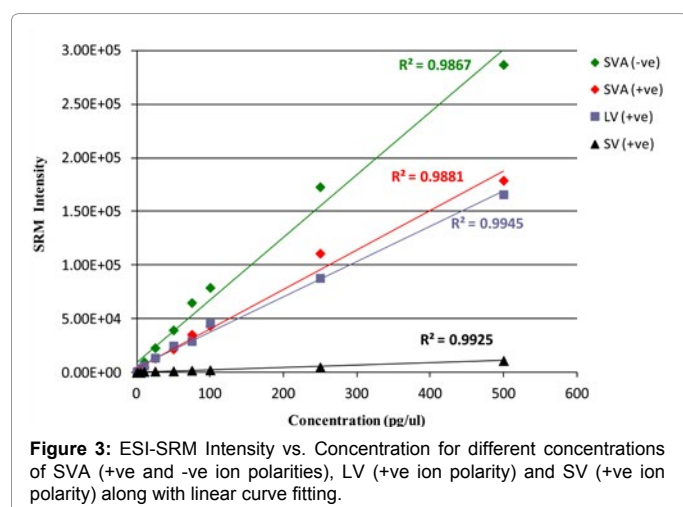


Figure 3: ESI-SRM Intensity vs. Concentration for different concentrations of SVA (+ve and -ve ion polarities), LV (+ve ion polarity) and SV (+ve ion polarity) along with linear curve fitting.

ions of SVA than SV. Figure 3 summarizes all the data of the three compounds depicted in the form of concentration vs. SRM intensity.

Discussion

The fragmentation characteristics of $[M+H]^+$ and $[M-H]^-$ of SVA under the conditions of turbo ionspray and that of $[M+H]^+$ of LV and SV in the ESI conditions have already been thoroughly elucidated [10,11]. The ESI daughter scan (MS/MS) spectra obtained herein (Figure S2) are in good agreement with those published reports. Therefore, the SRM transitions chosen in this investigation, by considering most intense daughter ion peaks (Table S2), are in accordance with the previous studies.

Defining molecular ESI Index (MESII)

The intensity of SRM chromatogram is directly related to the population of ions trapped in first quadrupole (Q1), i.e., population of precursor ions that are subjected to CID for SRM experiments. Therefore, this intensity can be considered as a measure of ion population in the gas phase that are electrosprayed from solution. Since known concentrations of the analyte are introduced into the mass spectrometer, the population of neutral molecules or ions in solution can be calculated; for which, we have used Avogadro's constant ($N_{Av} = 6.023 \times 10^{23}$) shown in Table S4. The intensity values determined from SRM (Table S3) were noted to be very much lower than the calculated in-solution population (Table S4), indicative of substantial loss of ions, as they move from spray region to Q1. The extent of such a loss is quite higher in the case of conventional ESI, when compared to nanospray [12,13]. Also, the amount of loss depends on the instrument configuration; for instance, recently Ibrahim, Smith and co-workers have designed and developed an ion funnel for efficient ion focusing, which has been shown to offer increased sensitivity [14]. Despite such losses, chemical property of the analyte would influence their ionic intensities recorded either by conventional mass spectrometry (i.e., molecular mass measurements) or tandem mass spectrometry (MS/MS).

We introduce a new parameter, 'molecular ESI index (MESII)', designated ϵ_{SRM} (see Equation 1), which we believe that it can be an indicator of degree of ionization of an analyte molecule.

$$\epsilon_{SRM} = -\log [\text{SRM Intensity} / \text{Population of ions or molecules in solution}] \dots (1)$$

Following equation (1), the SRM intensities (Table S3) were thus

used to calculate ϵ_{SRM} values for SVA (in positive and negative ion polarities) and SV and LV (in positive ion polarity). Because of applying negative logarithm in the equation, lower ϵ_{SRM} value will signify higher degree of ionization, which means that more ions have been detected in gas phase. ϵ_{SRM} values can help in understanding the efficiency of transfer of ions in solution to gas phase or efficiency of ionization of neutral molecules in-solution by means of electrospray; thereby ϵ_{SRM} gives an idea about extent of loss of ions that would occur as the ions move from spray region to Q1. Thus, the value of ϵ_{SRM} is an indicator of sensitivity of detection too, i.e., how much lower concentration of a particular compound could be detected. As an example, the difference that might arise between the ϵ_{SRM} values of a compound, when estimated from the recently developed instrument by Chen et al. [14] and from another type of mass spectrometer could provide hint about the variation in the sensitivity of detection of the two instruments.

Instrument configuration such as ion source geometry, collision cell geometry and ion-transfer optics significantly impact the process of detection and hence, ionic intensity. Off-axial or orthogonal sampling of electrospray and configuration of ion-transfer optics in the spectrometer are some factors that largely influence sensitivity [14,15]. Dependence of analyte ion's signal on their concentration, following ESI has been examined [15]. Not only concentration, the extent of ionization of analyte is significantly influenced by the matrix and other co-existing components also [15,16]. For instance, Wilm investigated the behaviour of electrospray ion signal with increasing analyte concentration and found suppression of hydrophilic analyte ion's signal by hydrophobic component present at high concentration in mixture [17]. Enke and co-workers had demonstrated the importance of relative gas-phase proton affinities of analyte and solvent, in understanding their relative response to ESI [18]. Furthermore, Hahne *et al.* showed enhancement in electrospray ionization of peptides by adding low percentages of dimethylsulfoxide (DMSO) in liquid chromatography solvents [19]. Therefore, ϵ_{SRM} of a molecule can indeed differ depending on the matrix conditions and instrumental settings. When ϵ_{SRM} of an analyte present in two different matrices are estimated using one kind of mass spectrometer, effect of matrix on the degree of ionization can be realized. Further, determining ϵ_{SRM} of an analyte present in a matrix, but using two different instruments, will help in knowing the influence of instrument configuration on the ionization of that analyte. Consequently, reporting a compound's ϵ_{SRM} must be accompanied by the instrumental and matrix properties that were utilized during the data acquisition. Here, the ϵ_{SRM} values for SVA, LV and SV are determined to be: ϵ_{SRM}^- (SVA): 7.2253, ϵ_{SRM}^+ (SVA): 7.4288, ϵ_{SRM}^+ (LV): 7.4541 and ϵ_{SRM}^+ (SV): 8.6833 (Table S3); it must be noted that these ϵ_{SRM} values have been calculated from the experiments that did not involve additives such as acetic acid or formic acid or DMSO in the solvents of LC. The same ϵ_{SRM} values may not be expected, while using the data recorded from another instrument of different configuration or when some additive is added either to the analytes or to LC solvents. Nevertheless, ϵ_{SRM} values determined under a set of conditions of an instrument may be extrapolated to other instruments as well. In other words, the trend observed in the variations of ϵ_{SRM} values between one and other analyte, i.e., ϵ_{SRM}^- (SVA) < ϵ_{SRM}^+ (SVA) < ϵ_{SRM}^+ (SV), can remain the same across different instruments, under constant/ fixed matrix conditions.

Furthermore, ϵ_{SRM} values can be useful in getting an idea about LOD, i.e., both instrument detection limit (IDL) as well as method detection limit (MDL), which are usually estimated based on S/N ratios. Signal intensity is related to the number of ions detected/generated, which depends on the degree of ionization of a particular analyte and ϵ_{SRM} is a measure of ionization extent under a given set of conditions. As already

discussed, a lower MESII value signifies higher ionization potential and therefore, a lower MESII could indicate lower LOD. For example, SVA could have a lower LOD, when characterized in negative ion polarity of ESI (ϵ_{SRM} (SVA): 7.2253) than detecting it in positive ion polarity (ϵ_{SRM} (SVA): 7.4288). Indeed, in negative ion polarity, SVA was detected till 500 fg μL^{-1} , S/N: 8.47; whereas in positive ion polarity, no good signals were observed below 1 pg μL^{-1} , whose S/N: 8.1 (Data not shown).

Interestingly, thus far, in the clinical investigations, only positive ion ESI-SRM has been extensively exploited for quantification of SV and LV [5,6]. To the best of our knowledge, negative ion ESI - SRM is yet to be implemented for estimating concentration of SVA in clinical studies. In fact, concentration determination of SVA is also crucial in clinical contexts, as SVA is the inhibitor of microsomal HMG-CoA reductase. We therefore believe that the ϵ_{SRM} values estimated herein can be useful indicators for quantification of these compounds in clinical and other contexts.

Additionally, it may be interesting to examine the method of 'selected ion monitoring (SIM)' for determining MESII value of a compound and compare it with ϵ_{SRM} , which might enable in comprehending the relative sensitivities of SIM and SRM methods. It is known that SIM does not involve CID and hence, a comparison of MESII deduced by SIM and ϵ_{SRM} might aid in understanding, how CID may contribute for sensitivity of detection. Also, for those molecules, which do not undergo CID properly, SIM can be used to estimate MESII.

Further, in the case polypeptides/proteins or oligonucleotides, whose ESI mass spectra are known to contain ensemble of peaks due to multiply charged ions, MESII needs to be calculated corresponding to every charge state, for which either SRM or SIM (or perhaps both) can be carried out, depending on the data quality. Such exercises on polypeptides and oligonucleotides will help in understanding the distribution of abundances of multiple charge states. And eventually, by means of MESII, it might be possible to predict or determine, which protein(s) or oligonucleotide(s) exhibit(s) higher or lower extent of ionization under a given conditions of ESI. Since SRM and SIM modes of detection have better sensitivity than conventional MS (i.e. full scan MS), MESII calculated from SRM/SIM intensities can be better than MESII determined from full scan MS intensities.

Conclusions

In the case of estimation of gas phase acidity and proton affinity/gas phase basicity by Cooks' kinetic method, a standard reference compound (SRC) is utilized. By suitably tuning the ionization source parameters, SRC is allowed to form proton bound dimer (cluster) ions with the analyte molecule of interest and the resulting cluster ion is subjected to CID [1]. Therefore, the values of gas phase acidity or proton affinity of an analyte are dependent on the gas phase acid/base character of SRC as well. However, herein, the MESII (ϵ_{SRM}) value of an analyte is estimated by subjecting only the analyte's molecular ion (for e.g., $[\text{M}+\text{H}]^+$ or $[\text{M}-\text{H}]^-$) to CID, by means of SRM experiments. Even though the definition of MESII (ϵ_{SRM}) does not involve any thermodynamic parameters, we believe that ϵ_{SRM} too can be considered as a gauge to assess the extent of ionization of molecules under the ESI conditions. Probably, ϵ_{SRM} value may provide a rough and somewhat a quick approximation about an analyte's response to ESI, prior to performing experiments of kinetic method. Perhaps, it might even be possible to correlate ϵ_{SRM} of a compound with the gas phase acidity and proton affinity/gas phase basicity values of that compound, under appropriate instrumental conditions; which needs to be examined. Additionally, ϵ_{SRM} values can also be estimated for adduct ions like

$[\text{M}+\text{Na}]^+$, $[\text{M}+\text{CH}_3\text{NH}_3]^+$ etc. Such determined ϵ_{SRM} values would offer clues as to, which analyte adduct ion shows better response to ESI and hence, ϵ_{SRM} value can be useful in choosing a better precursor ion for SRM experiments.

Furthermore, once ϵ_{SRM} value of a compound is well established under a set of conditions (i.e., matrix and instrumentation), then it might become possible to use that value for quantitating that compound, provided the quantitation experiments are carried out under the same conditions, which were followed for deduction of ϵ_{SRM} .

Acknowledgements

VS is thankful to Dr. Rajesh S. Gokhale, Director, CSIR-IGIB for support and encouragement. RG is thankful to Ritika Goyal and Rikeshwer Dewangan for their support and assistance. RG wishes to express her thanks to Deepshikha Shrivastava (M.Sc. trainee), who put initial efforts for optimizing LC-MS conditions, which was done for her M.Sc. dissertation work under the guidance of VS at CSIR-IGIB. Project N1262 of AIIMS, New Delhi is gratefully acknowledged for providing fellowship to RG.

The preliminary results of this work were presented as a poster in the Analytica Acta-2012 (3rd International Conference and Exhibition on Analytical and Bioanalytical Techniques), which was held during November 22-24, 2012 at Hyderabad International Convention Centre, Hyderabad, India.

References

1. Cooks RG, Patrick JS, Kotiaho T, McLuckey SA (1994) Thermochemical determinations by the kinetic method. *Mass Spectrom Rev* 13: 287-339.
2. Ren J, Patel CG (2005) Determination of the gas-phase acidity of methylthioacetic acid using the Cooks' kinetic method. *J Am Soc Mass Spectrom* 16: 535-541.
3. Tan JP, Ren J (2007) Determination of the gas-phase acidities of cysteine-polyalanine peptides using the extended kinetic method. *J Am Soc Mass Spectrom* 18: 188-194.
4. Miao XS, Metcalfe CD (2003) Determination of cholesterol-lowering statin drugs in aqueous samples using liquid chromatography-electrospray ionization tandem mass spectrometry. *J Chromatogr A* 998: 133-141.
5. Lilja JJ, Kivisto KT, Neuvonen PJ (1998) Grapefruit juice-simvastatin interaction: Effect on serum concentrations of simvastatin, simvastatin acid, and HMG-CoA reductase inhibitors. *Clin Pharmacol Ther* 64: 477-483.
6. Mousa O, Brater DC, Sundblad KJ, Hall SD (2000) The interaction of diltiazem with simvastatin. *Clin Pharmacol Ther* 67: 267-274.
7. Rao PN, Mullangi R, Kuamr IJ, Kumari NI, N Seshagiri Rao JVL (2012) Simultaneous determination of simvastatin, lovastatin and niacin in human plasma by LC-MS/MS and its application to a human pharmacokinetic study. *Biomed Chromatogr* 26: 476-484.
8. Moron B, Verma AK, Das P, Taavela J, Dafik L, et al. (2013) CYP3A4-Catalyzed simvastatin metabolism as a non-invasive marker of small intestinal health in celiac disease. *Am J Gastroenterol* 108: 1344-1351.
9. Vree TB, Dammers E, Ulc I, Horkovics-Kovats S, Ryska M, et al. (2003) Differences Between Lovastatin and Simvastatin Hydrolysis in Healthy Male and Female Volunteers: Gut hydrolysis of lovastatin is twice that of simvastatin. *Scientific World Journal* 3: 1332-1343.
10. Zhao JJ, Xie IH, Yang AY, Roadcap BA, Rogers JD (2000) Quantitation of simvastatin and its β -hydroxy acid in human plasma by liquid-liquid cartridge extraction and liquid chromatography/tandem mass spectrometry. *J Mass Spectrom* 35: 1133-1143.
11. Wang H, Wu Y, Zhao Z (2001) Fragmentation study of simvastatin and lovastatin using electrospray ionization tandem mass spectrometry. *J Mass Spectrom* 36: 58-70.
12. Smith RD, Shen Y, Tang K (2004) Ultrasensitive and quantitative analyses from combined separations-Mass Spectrometry for characterization of proteomes. *Acc Chem Res* 37: 269-278.
13. Arnaud CH (2006) Mass spec tackles proteins. *Chem Eng News* 84: 17-25.
14. Chen TC, Fillmore TL, Prost SA, Moore RJ, Ibrahim YM, et al. (2015) Orthogonal Injection Ion Funnel Interface Providing Enhanced Performance

-
- for Selected Reaction Monitoring-Triple Quadrupole Mass Spectrometry. *Anal Chem* 87: 7326-7331.
15. Cech NB, Enke CG (2001) Practical implications of some recent studies in electrospray ionization fundamentals. *Mass Spectrom Rev* 20: 362-387.
16. Tang L, Kebarle P (1993) Dependence of ion intensity in electrospray mass spectrometry on the concentration of the analytes in the electrosprayed solution. *Anal Chem* 65: 3654-3668.
17. Wilm M (2011) Principles of electrospray ionization. *Mol Cell Proteom* 10: 1-8.
18. Amad MH, Cech NB, Jackson GS, Enke CG (2000) Importance of gas-phase proton affinities in determining the electrospray ionization response for analytes and solvents. *J Mass Spectrom* 35: 784-789.
19. Hahne H, Pachi F, Ruprecht B, Maier SK, Kläeger S, et al. (2013) DMSO enhances electrospray response, boosting sensitivity of proteomic experiments. *Nat Methods* 10: 989-991.