8. Příloha

8.1 Seznam příloh

- Comparison of manual and semiautomatic approaches of MEPS as sample preparation technique in UHPLC-MS/MS methods for determination of statins in biological samples *(H. Vlčková, V. Pilařová, P. Svoboda, L. Nováková, P. Solich)*
  Informal Meeting on Mass Spectrometry, Olomouc, Česká republika; 2012

- Development of multistatin method for determination of statins, their metabolites and interconversion products in human serum *(H. Vlčková, K. Škrášková, P. Svoboda, H. Tomšíková, P. Solich, L. Nováková)*
  International Mass Spectrometry Conference, Kjóto, Japonsko; 2012

- Development of UHPLC-MS/MS method for the determination of statins and their metabolites *(P. Svoboda, H. Vlčková, L. Nováková)*
  Česká chromatografická škola – HPLC 2013, Seč, Česká republika; 2013

Comparison of manual and semiautomatic approaches of MEPS as sample preparation technique in UHPLC-MS/MS methods for determination of statins in biological samples

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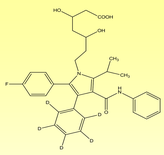
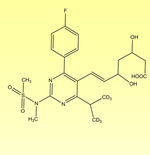
Introduction

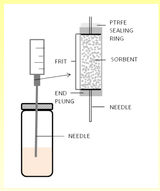


ATORVASTATIN



ROSUVASTATIN

Statins are inhibitors of 3-hydroxy-3-methylglutaryl (HMG) coenzyme A (CoA) reductase. They belong among the hypolipidemic drugs widely used for the treatment of hypercholesterolemia. They reduce the total cholesterol, LDL-cholesterol and significantly decrease a risk of atherosclerosis and mortality and morbidity associated with coronary heart disease. Atorvastatin and rosuvastatin are the most often used statins in clinical practices.
Statin molecules exist in two forms, open- ring hydroxy acid and lactone forms. An interconversion between open -ring hydroxy acid and lactone forms occurs in vivo but during analysis of samples too. For this reason the suitable conditions to eliminate or minimize interconversion have to be selected.
In contrast to "fast LC", conventional sample preparation approaches such as solid phase extraction are still high labor intensive and time-consuming. Therefore microextraction by packed sorbent (MEPS) was chosen as the suitable sample preparation technique. MEPS as a miniaturized SPE is logical extension of SPE for the analysis of biological fluids. It is implemented by needle and syringe containing 1- 2 mg of sorbent in the barrel of syringe. Sample preparation takes place on the packed bed. The process can be manual, semi-automatic or fully automatic and on-line connected with LC.



The aim of the work

The aim of this work was to develope and validate two sensitive and selective UHPLC-MS/MS methods for determination of statins in biological samples. The MEPS was selected as an appropriate sample preparation technique, because it is fast, simple and effective method used small volumes of sample and solvents. The first one for determination of atorvastatin, and its derivates in human serum employs the manual approach of MEPS and the second one for the determination atorvastatin, rosuvastatin and their derivates employs the semi-automatic approach of MEPS. The comparison manual and semi-automatic approaches was performed. Both method are used for the monitoring of atorvastatin and rosuvastatin concentrations in many biological samples in clinical practice.

UHPLC-MS/MS methods

INSTRUMENTS: Acquity UPLC (ACQ-BSM, ACQ SM, ACQ DAD, ACQ CM), Waters
Quattro Micro triple quadropole mass spectrometer, Waters

For the both methods ESI ionisation was used. The quantification of analytes was performed using SRM experiments for each analytes. Deuterium labeled standards were employed for this measurement. Cone voltage and collision energy were set up individually for the each analyte.

The determination of atorvastatin and its derivates in human serum

UHPLC conditions

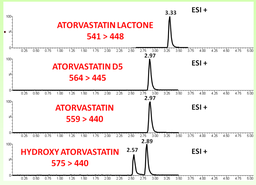
Analytical column: Acquity BEH C18 (2.1 x 100 mm, 1.7 um)
Mobile phase: 0.5 mM AmAc pH 4.0:ACN gradient elution
Flow-rate: 0.25 ml/min
Injected volume: 5 µl
Column temperature: 30°C
Autosampler temperature: 4°C

MS/MS conditions

ESI$^+$

Capillary voltage	[kV]	3.5
Extractor	[V]	3
RF lens (hexapole)	[V]	1.0
Source temperature	[°C]	130
Desolvation temperature	[°C]	375
Gas flow – desolvation	[l/hod]	500
Gas flow – cone	[l/hod]	120

Chromatogram of standards



compounds	ionisation mode	precursors	fragments	dwell time	cone V
p-OH-AT	ESI +	575.0	440.1	0.05	30
			466.2		30
o-OH-AT	ESI +	575.0	440.1	0.05	30
			466.2		30
AT	ESI +	559.0	440.1	0.05	30
			466.1		30
AT-D5	ESI +	564.0	445.1	0.05	30
			471.0		30

The determination of atorvastatin, rosuvastatin and their derivates in human serum

UHPLC conditions

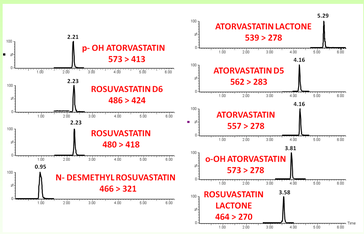
Analytical column: Acquity BEH C18 (2.1 x 50 mm, 1.7 um)
Mobile phase: 0,5 mM AmAc pH 4.0:ACN gradient elution
Flow-rate: 0.30 ml/min
Injected volume: 5 µl
Column temperature: 30°C
Autosampler temperature: 4°C

MS/MS conditions

ESI$^+$/$^-$

Capillary voltage	[V]	2.5
Extractor	[V]	1
RF lens (hexapole)	[V]	0.5
Source temperature	[°C]	130
Desolvation temperature	[°C]	450
Gas flow – desolvation	[l/hod]	650
Gas flow – cone	[l/hod]	70

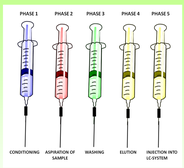
Chromatogram of standards



compounds	ionisation mode	prekursors	fragments	dwell time	cone V	collision E	t_R
RV	ESI -	479.8	418.2	0.10	30	15.0	2.26
DM-RV	ESI -	465.8	404.1	0.12	30	15.0	0.92
RV-L	ESI +	463.9	270.3	0.05	50	35.0	3.57
RV-D6	ESI -	485.8	424.2	0.05	30	15.0	2.23
p-OH-AT	ESI -	573.1	413.1	0.1	35	30.0	2.23
o-OH-AT	ESI -	573.1	287.3	0.05	40	40.0	3.84
AT	ESI -	557.0	278.3	0.07	40	40.0	4.22
AT-D5	ESI -	562.1	283.3	0.05	40	40.0	4.18
AT-L	ESI -	539.2	278.2	0.05	30	40.0	5.27

Sample preparation - MEPS & SPE

MEPS PROCEDURE



SPE

analytes: atorvastatin, atorvastatin lactone, p-OH atorvastatin, o-OH atorvastatin
matrix: human serum
sorbent: Discovery DSC-18

procedure:
1. 1ml ACN
2. 1 ml 0.1 M AmAc pH 4.5
3. 0.5 ml of SAMPLE
4. 1 ml ACN/ 0.01 M AmAc pH 4.5 (15/85)
5. 1 ml ACN/0.1 M AmAc pH 4.5 (95/5)

MANUAL APPROACH of MEPS

analytes: atorvastatin, atorvastatin lactone, p-OH atorvastatin, o-OH atorvastatin
matrix: human serum
sorbent: MEPS C8

procedure:
1. 3x 100 µl ACN
2. 3x 100 µl 0.1 M AmAc pH 4.5
3. 50 µl of SAMPLE
4. 2x 100 µl 0.1 M AmAc pH 4.5
5. 100 µl ACN/ 0.01 M AmAc pH 4.5 (15/85)
6. 100 µl ACN/0.1 M AmAc pH 4.5 (95/5)

SEMI– AUTOMATIC APPROACH of MEPS

analytes: atorvastatin, atorvastatin lactone, p-OH atorvastatin, o-OH atorvastatin, rosuvastatin, rosuvastatin lactone, N-desmethyl rosuvastatin
matrix: human serum
sorbent: MEPS C8

procedure:
1. 3x 100 µl ACN
2. 3x 100 µl 0.1 M AmAc pH 4.5
3. 50 µl of SAMPLE
4. 2x 100 µl 0.1 M AmAc pH 4.5
5. 100 µl ACN/ 0.01 M AmAc pH 4.5 (5/95)
6. 100 µl ACN/0.1 M AmAc pH 4.5 (95/5)

Comparison of manual and semi– automatic MEPS procedures

ADVANTAGE of MEPS:

- less time- consuming
- simplicity
- multiple re-use of sorbent
- low consumption of sample and solvents
- maintaining of sufficient efficiency, accuracy and precision

	SPE		MEPS – manually		MEPS – semi- automatic	
	AT	ATL	AT	ATL	AT	ATL
Linearity	0.9994	0.9995	0.9993	0.9993	0.9998	0.9990
Accuracy	84 – 86%	78 – 93%	101 – 115%	89 – 111%	100 – 110%	93 – 101%
Precision	< 7%	< 11%	< 2%	< 5%	< 3%	< 6%
Matrix effects	-	-	95 – 102%	93 – 106%	98 – 102%	94 – 100%

	SPE	MEPS – manually	MEPS – semi- automatic
time of extraction	20 min	7 min	5 min
volume of solvent	9 ml	1,3 ml	1,3 ml
volume of sample	500 µl	50 µl	50 µl
re-use	1 – 5x	100x	100x
evaporation	yes	no	no

DISADVANTAGE of MANUAL APPROACHES of MEPS

- dependence of analyte recoveries on the continual movement of plunger and on rate of sample passing through the sorbent
- lower precision of aspiration of small sample volumes (< 50µl)
- high requirements on the operators

THESE DISADVANTAGES ARE REMOVED BY USING THE SEMI-AUTOMATIC APPROACH

AUTOMATIC APPROACH of MEPS as the most suitable mode of MEPS

- automation → minimal influence of operator → posibility of on-line connection with LC system
- high precision of injection of small sample volumes (10µl)
- low volume of sample (< 10µl)
- a rate of MEPS extraction

Conclusions

Two fast, sensitive and selective methods were developed for the determination of statins by UHPLC-MS/MS in human serum. Microextraction by packed sorbent in manual and semi-automated approaches was used as sample preparation technique. The both methods were used in clinical practice for the determination of atorvastatin and rosuvastatin and their metabolites in serum samples of patients with familiar hypercholesterolemia treated by statins. The semi-automatic MEPS approach is more suitable for the analyses of biological sample, because it enables continual movement, more precise aspiration of small sample volume and it is easier for operator than manual approach. The fully automatic mode MEPS is the most appropriate for analysis of large number of samples in routine clinical laboratories.

Acknowledgements

The authors gratefully acknowledge the financial support of research projects UNCE 17/2012.

DEVELOPMENT OF MULTISTATIN METHOD FOR DETERMINATION OF STATINS, THEIR METABOLITES AND INTERCONVESION PRODUCTS IN HUMAN SERUM

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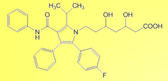
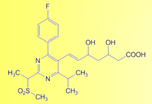
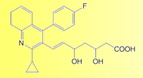
ATORVASTATIN



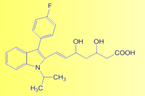
ROSUVASTATIN



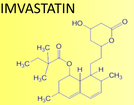
PITAVASTATIN



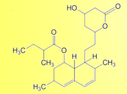
FLUVASTATIN



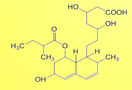
SIMVASTATIN



LOVASTATIN



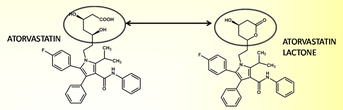
PRAVASTATIN



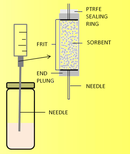
Introduction

Statins are inhibitors of 3-hydroxy-3-methylglutaryl (HMG) coenzyme A (CoA) reductase. They belong among the hypolipidemic drugs widely used for the treatment of hypercholesterolemia. They reduce the total cholesterol, LDL-cholesterol and significantly decrease a risk of atherosclerosis, mortality and morbidity associated with coronary heart disease. Recently, anticancer effect has been discovered for several statins.

Statin molecules exist in two forms, open- ring hydroxy acid and lactone forms. An interconversion between open-ring hydroxy acid and lactone forms occurs in vivo but during analysis of samples too. For this reason suitable conditions to eliminate or minimize the interconversion have to be selected. Both forms have to be determined together.



In contrast to "fast LC chromatography approaches", conventional sample preparation technique, such as solid phase extraction are still high labor- intensive and time-consuming. Therefore microextraction by packed sorbent (MEPS) was chosen as a suitable sample preparation technique. MEPS is miniaturized SPE and has the same principle. It is implemented by needle and syringe containing 1- 2 mg of sorbent in the barrel of syringe. Sample preparation takes place on the packed bed.



The process can be manual, semi-automatic or fully automatic and on-line coupled with LC.

The aim of the work

The aim of this project was to develop and validate UHPLC-MS/MS method and convenient sample preparation procedure for simultaneous determination of statins (atorvastatin, rosuvastatin, simvastatin, lovastatin, pravastatin, fluvastatin and pitavastatin), their metabolites and interconversion products in human serum. The MEPS was selected as an appropriate sample preparation technique, because it is fast, simple and effective method using small volumes of sample. The validated method will be used for the monitoring of statines in human serum.

Analytical method

INSTRUMENTS: Acquity UPLC (ACQ-BSM ACQ SM, ACQ DAD, ACQ CM), Waters
Quattro Micro triple quadropole mass spectrometer, Waters

UHPLC conditions

Analytical column:	Acquity BEH C_{18} (100 × 2.1 mm, 1.7 µm)
Mobile phase:	ACN: 0.5mM AmAc pH 4.0 Gradient elution
Flow-rate:	0.30 ml/min
Injection volume:	5 µl
Weak wash:	20% ACN
Analysis time:	10.5 min

ESI MS/MS conditions

Ionization type:	polarity switching	
Capillary voltage	[V]	2.5
Extractor	[V]	1.0
RF lens (hexapole)	[V]	0.5
Source temperature	[°C]	130 °C
Desolvation temperature	[°C]	450 °C
Gas flow – desolvation	[l/hod]	650 l/hr
Gas flow – cone	[l/hod]	70 l/hr

RESULTS

Compounds	precursor	fragment	cone V	collision E	mode	t_R (min)
P-OH Atorvastatin (p-OH AT)	573.1	413.1	35	30	ESI-	2.19
O-OH Atorvastatin (o-OH AT)	573.1	278.3	40	40	ESI-	3.85
Atorvastatin (AT)	557.0	278.3	40	40	ESI-	4.21
Atorvastatin lactone (ATL)	539.2	278.2	30	40	ESI-	5.35
N-Desmethyl Rosuvastatin (DM-RV)	465.8	404.1	30	15	ESI-	0.84
Rosuvastatin (RV)	479.8	418.2	30	15	ESI-	2.19
Rosuvastatin lactone (RVL)	463.9	270.3	50	35	ESI+	3.63
Pitavastatin (PTV)	419.8	358.1	25	15	ESI-	3.28
Pitavastatin lactone (PTVL)	403.9	290.2	45	30	ESI+	5.18
Fluvastatin (FV)	410.1	348.1	25	15	ESI-	4.13
Fluvastatin lactone (FVL)	394.1	376.1	25	15	ESI+	5.73
Simvastatin acid (SVA)	434.9	319.2	30	15	ESI-	5.54
Simvastatin (SV)	419.0	285.1	20	10	ESI+	7.21
Lovastatin acid (LVA)	420.9	319.2	25	15	ESI-	4.79
Lovastatin (LV)	405.0	285.3	20	10	ESI+	6.42
Pravastatin (PV)	422.9	321.3	30	15	ESI-	1.13
Pravastatin lactone (PVL)	407.0	183.2	20	15	ESI+	2.07

The quantification of analytes was performed using SRM experiments for each analytes.

Cone voltage and collision energy were set up individually for the each analytes.

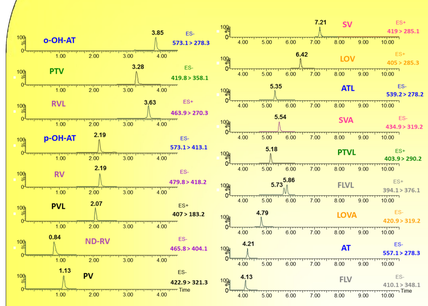
Deuterium labeled standards (PV-d_3, PVL -d_3, RV-d_6, RVL-d_6, PTV-d_5, PTVL-d5, AT-d5, o-OH AT-d5, ATL-d5, LVA-d3, SVA-d6, SV-d6) were employed for this measurement.

Sample preparation - MEPS method

PROCEDURE		
conditioning	3x100 µl	ACN
conditioning	3x100 µl	0.1M AmAc pH 4.5
sampling	50 µl	sample
washing	100 µl	0.1 M AmAc pH 4.5
	100 µl	ACN/0.01M AmAc pH 4.5 (05/95)
elution	100 µl	ACN/0.1M AmAc pH 4.5 (95/5)

MEPS C8 sorbent was used. Primarily MEPS conditions were optimized using the standard solution and subsequently on human serum. Suitable types and volumes of sample, washing and elution solvents were found. The mixture of acetonitrile and 0.1M ammonium acetate buffer pH 4.5 was used as the elution solvent. In order to obtain high recovery 50 µl of sample and 100 µl elution solvent was applied. The mixture of ACN and buffer (5/95) was used as the washing solvent . More than 5% of organic component causes elution more polar analytes in washing step.

Chromatogram of standards



Validation data

Analytes	linearity (r^2)	RSD- t_R [%]	RSD- t_R [%]	LOQ [ng/ml]	mode
p-OH-AT	0.9994	0.11	2.51	2.5	ESI -
o-OH-AT	0.9997	0.20	2.19	2.5	ESI -
AT	0.9997	0.10	2.05	2.5	ESI -
ATL	0.9991	0.07	3.86	2.5	ESI -
DM-RV	0.9996	0.25	3.29	1	ESI -
RV	0.9996	0.13	1.34	2.5	ESI -
RVL	0.9995	0.13	3.17	2.5	ESI +
PV	0.9993	0.00	2.08	2.5	ESI -
PVL	0.9986	0.37	4.79	10	ESI +
SV	0.9994	0.07	1.85	2.5	ESI +
SVA	0.9996	0.05	1.84	25	ESI -
LV	0.9991	0.08	2.36	2.5	ESI +
LVA	0.9974	0.08	2.51	10	ESI -
FLV	0.9996	0.00	2.36	2.5	ESI -
FLVL	0.9995	0.09	5.31	2.5	ESI +
PTV	0.9994	0.10	1.36	1	ESI -
PTVL	0.9997	0.13	1.78	2.5	ESI +

The SST was performed by 10 subsequent injections of standard mixture of measured analytes at the concentration 500, 100 and 10 ng/ml, example is shown for the concentration 10 ng/ml. Linearity of calibration curves was measured in the concentration range 1– 500 ng/ml, LOD and LOQ were calculated based on S/N ratio: S/N = 3 for LOD and S/N = 10 for LOQ.

Conclusion

A new UHPLC-MS/MS method for determination of several statines, their metabolites and interconversion products (in total 17 analytes) was developed. 12 deuterium labeled standards were employed for the quantification.

MEPS was used as the sample preparation method because it is fast, simple using the low volume of samples and solvents. Individual analytes are different in their polarity, therefore the choice of suitable washing and elution solvents for MEPS procedure were complicated. The extraction process was developed on the standard solutions, subsequently it will be optimized on the human serum.

MEPS-UHPLC-MS/MS method will be validated and applied to the real samples.

Acknowledgements

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INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Development of UHPLC-MS/MS method for the determination of statins and their metabolites

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Introduction and the aim of the work

Statins are the most important cholesterol-lowering drugs. These hypolipidemics rank among the most frequently prescribed drugs in a clinical practice in the Czech Republic. The mechanism of action of statins is based on the inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, which is the key enzyme of cholesterol synthesis. Statins significantly reduce the plasma level of total and LDL-cholesterol which decreases the risk of morbidity and mortality.

The prodrug (lactone) and active form (open hydroxy-acid) of individual statins must be monitored simultaneously as the interconversion between the two is observed both *in vivo* and *in vitro* (Figure 1).

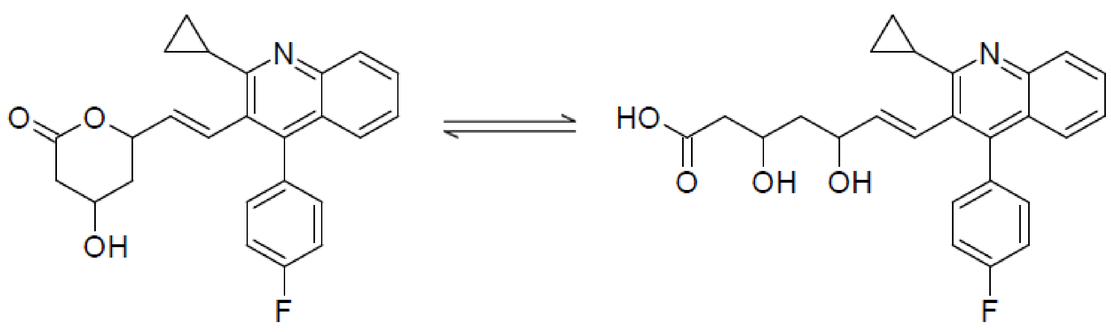

Figure 1: Interconversion between lactone (prodrug) and acid (effective) form of pitavastatin

The aim of the work was to develop a sensitive UHPLC-MS/MS method for the determination of statins and their metabolites. The total number of examined analytes was 17. First, the chromatographic conditions were optimized. Next, the optimization of the mass spectrometry parameters was performed. Finally, the linearity, sensitivity and repeatability of the method were measured.

UHPLC conditions

The optimization of chromatographic conditions included the selection of the stationary phase, mobile phase and the conditions of gradient elution. Analytical column **Acquity BEH C18 column (2.1 x 50 mm; 1.7µm)** was used for the separation (Figure 2). The mobile phase was consisted of 0.1 mmol/l ammonium acetate pH = 4 and acetonitrile. Gradient elution was performed according to Table 1.

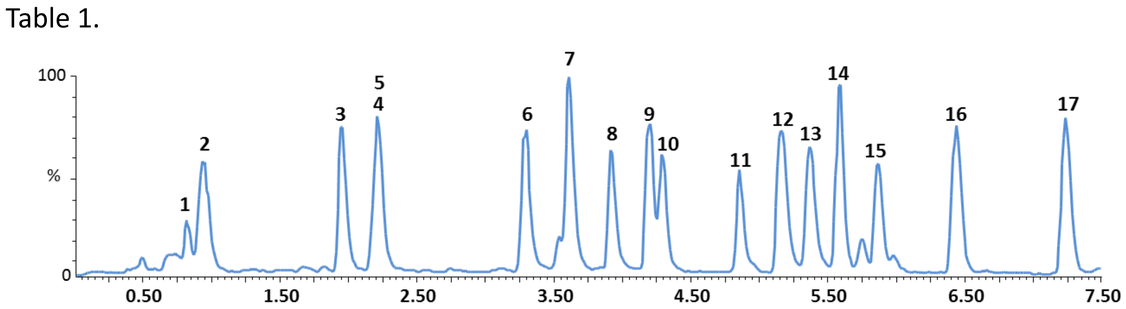

Figure 2: Gradient elution optimization:

1 – N-Desmethylrosuvastatin, 2 – Pravastatin, 3 – Pravastatin lactone, 4 – Rosuvastatin, 5 – *p*-Hydroxyatorvastatin, 6 – Pitavastatin, 7 – Rosuvastatin lactone, 8 – *o*-Hydroxyatorvastatin, 9 – Fluvastatin, 10 – Atorvastatin, 11 – Lovastatin acid, 12 – Pitavastatin lactone, 13 – Atorvastatin lactone, 14 – Simvastatin acid, 15 – Fluvastatin lactone, 16 – Lovastatin, 17 – Simvastatin

Time	Flow [ml/min]	0.1 mM AmAc pH = 4 [%]	Acetonitrile [%]	Curve
initial	0.300	70.0	30.0	Initial
7.00	0.300	35.0	65.0	6
7.50	0.300	70.0	30.0	6
10.50	0.300	70.0	30.0	6

Table 1: Conditions of gradient elution (Acquity Ultra Performance LC - Waters)

MS/MS conditions

First, the precursor ion was selected. Then, the ion source parameters were optimized (Table 2). The next step included the selection of four product ions. All the three operations were performed in both ionization modes. After that, the ion transitions (Figure 3) were optimized and the most intensive one with appropriate collision energy was selected for the majority of the analytes in both ionization modes. The resulting ionization mode (Table 3) for the statin molecule was chosen on the basis of peak area at $5x10^{-7}$ g/ml, calibration curve linearity and limit of quantification based on S/N approach.

Ion source optimization

Ion source	ESI^+	ESI^-	ESI^+/ESI^-
Capillary voltage	3.0 kV	2.5 kV	2.5 kV
Extractor voltage	2.0 V	1.0 V	1.0 V
Hexapole voltage	0.0 V	0.7 V	0.5 V
Source temperature	130 °C	130 °C	130 °C
Desolvation temperature	425 °C	450 °C	450 °C
Desolvation gas flow	650 l/h	600 l/h	600 l/h
Cone gas flow	85 l/h	60 l/h	70 l/h

Table 2: Optimized ion source parameters

MRM chromatogram

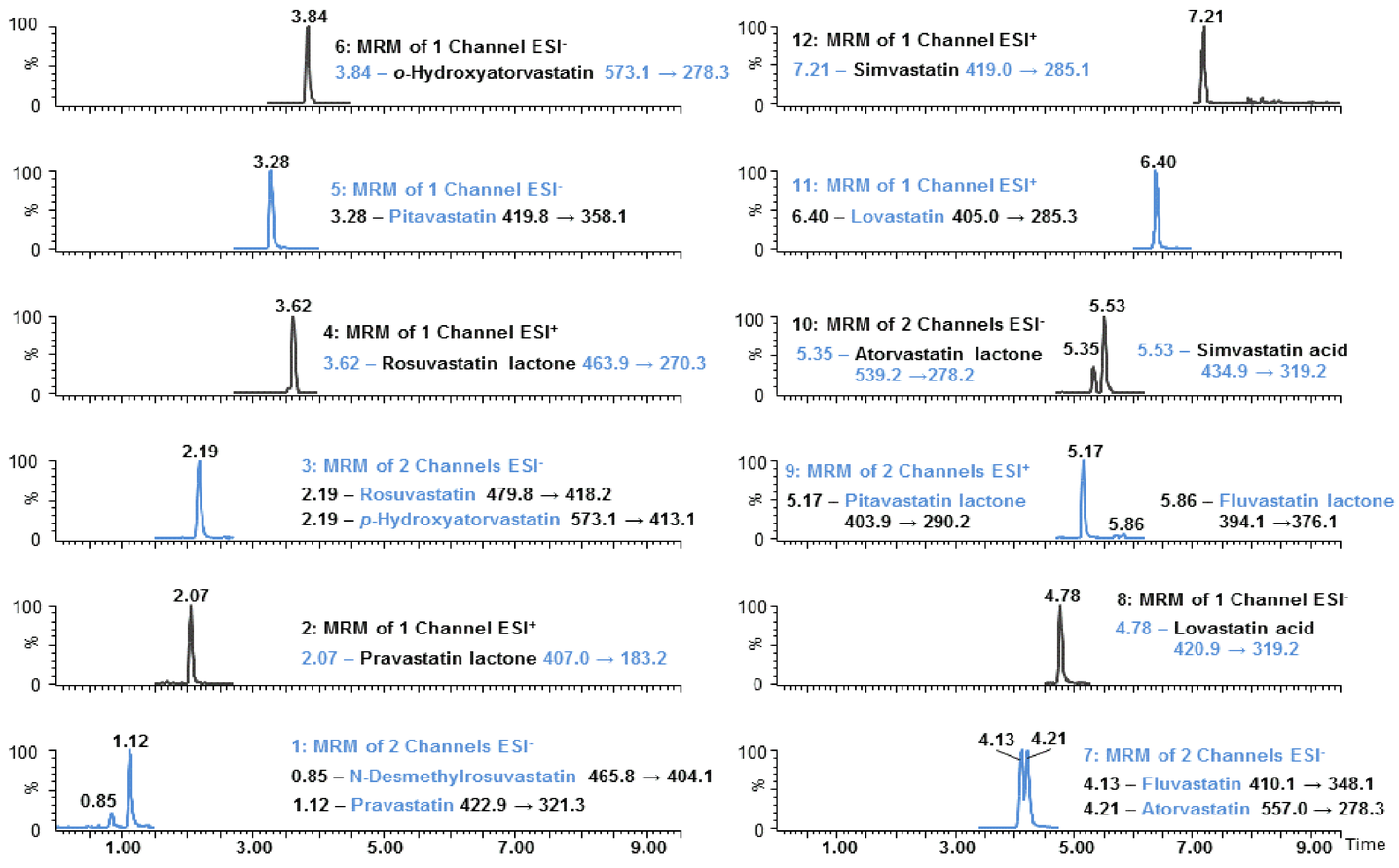

Figure 3: Chromatogram of multiple reaction monitoring

Ion transitions optimization

Compound	Ionization mode	Ion transition	Dwell time	Cone voltage [V]	Collision energy [V]	Time window [min]	LOQ [g/ml]
Pravastatin	ESI^-	422.9 → 321.3	0.120	30	15	0 - 1.5	$2.5x10^{-9}$
N-Desmethylrosuvastatin	ESI^-	465.8 → 404.1	0.120	30	15	0 - 1.5	$1x10^{-9}$
Pravastatin lactone	ESI^+	407.0 → 183.2	0.100	20	15	1.5 - 2.7	$1x10^{-8}$
Rosuvastatin	ESI^-	479.8 → 418.2	0.100	30	15	1.5 - 2.7	$2.5x10^{-9}$
p-Hydroxytorvastatin	ESI^-	573.1 → 413.1	0.100	35	30	1.5 - 2.7	$2.5x10^{-9}$
Pitavastatin	ESI^-	419.8 → 358.1	0.050	25	15	2.7 - 4.0	$1x10^{-9}$
Rosuvastatin lactone	ESI^+	463.9 → 270.3	0.050	50	35	2.7 - 4.0	$2.5x10^{-9}$
o-Hydroxytorvastatin	ESI^-	573.1 → 278.3	0.050	40	40	3.2 - 4.5	$2.5x10^{-9}$
Fluvastatin	ESI^-	410.1 → 348.1	0.070	25	15	3.5 - 4.65	$2.5x10^{-9}$
Atorvastatin	ESI^-	557.0 → 278.3	0.070	40	40	3.5 - 4.65	$2.5x10^{-9}$
Lovastatin acid	ESI^-	420.9 → 319.2	0.050	25	15	4.5 - 5.3	$1x10^{-8}$
Pitavastatin lactone	ESI^+	403.9 → 290.2	0.050	45	30	4.7 - 6.2	$2.5x10^{-9}$
Simvastatin acid	ESI^-	434.9 → 319.2	0.050	30	15	4.7 - 6.2	$1x10^{-9}$
Atorvastatin lactone	ESI^-	539.2 →278.2	0.050	30	40	4.7 - 6.2	$2.5x10^{-9}$
Fluvastatin lactone	ESI^+	394.1 →376.1	0.050	25	15	4.7 - 6.2	$2.5x10^{-9}$
Lovastatin	ESI^+	405.0 → 285.3	0.200	20	10	6.0 - 7.0	$2.5x10^{-9}$
Simvastatin	ESI^+	419.0 → 285.1	0.200	20	10	7.0 - 10.5	$2.5x10^{-9}$

Table 3: Optimized ion transitions

Conclusions

UHPLC-MS/MS method for determination of 17 statin molecules was developed and provided good repeatability. RSD for peak areas at $1x10^{-8}$ g/ml was between 1.53 % and 8.52 % and RSD for retention time at the same concentration between 0.00 % and 0.72 %. RSD for peak areas at $1x10^{-7}$ g/ml ranged between 0.62 % and 4.10 % and RSD for retention time at the same concentration between 0.00 % and 0.12 %. These values were obtained at optimized conditions using ESI polarity switching mode. The LOQ value was in the range $1x10^{-9}$ - $1x10^{-8}$ g/ml and the linearity was ≥ 0.9990 for 15 analytes and > 0.9900 for 2 remaining analytes (lovastatin acid and pravastatin lactone).

Acknowledgements

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