Development and validation of a QTrap method for sensitive quantification of sphingosine 1-phosphate

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Abstract
Sphingosine 1-phosphate (S1P) is a bioactive phospholipid and ligand for five G protein-coupled cell-surface receptors designated S1PR1–5. The determination of low levels of S1P remains a challenge and usually requires sophisticated analytical instrumentation and methodology. This report describes a technique using the linear ion trap mode of a basic QTrap triple-quadrupole mass spectrometer. S1P was extracted from acidified biological samples using a modified Folch extraction procedure. After the addition of C17-sphingosine as an internal standard, a step gradient LC method was used to separate the analytes on a reversed-phase C18 MultoHigh analytical column. After the internal standard C17-sphingosine was detected by multiple reaction monitoring (MRM), the detection mode was switched to enhanced product ion (EPI) mode for the detection of S1P. The mode was switched back to MRM again for the detection of other analytes. Using this QTrap method, we reached a limit of detection of 1 nM and a limit of quantification of 3 nM for S1P, which was up to 30 times more sensitive than the MRM mode with the same instrument. Intra-day precision ranged between −3.8 and 6.3%, and inter-day precision was between −13.8 and 3.3%, depending on the spiked S1P concentration.

KEYWORDS
enhanced product ion, LC, S1P, sphingolipid, triple-quadrupole MS

1 | INTRODUCTION

Sphingosine 1-phosphate (S1P) is a low-abundant sphingolipid metabolite. It is present at high concentrations in blood and lymph. Reported human plasma values are in the range of 200–800 nM (Andreani & Graler, 2006; He, Huang, & Schuchman, 2009; Schmidt, Schmidt, & Geisslinger, 2006), whereas serum levels are usually in the low micromolar range (Andreani & Graler, 2006; Winkler et al., 2015). Red blood cells are a major source for S1P in circulation and contain 1.5–3 nmol S1P per 1 × 1012 ghosts (Bode et al., 2010; Sun et al., 2016). In tissues, S1P concentrations are typically low due to the constitutively active S1P-degrading enzyme S1P-lyase, which cleaves S1P irreversibly into hexadecenal and phosphoethanolamine. Reported values, however, differ significantly with values ranging from 0.75 to 28 μmol/kg lung tissue, similar for other tissues (He, Huang, & Schuchman, 2009; Hisano, Kobayashi, Yamaguchi, & Nishi, 2012). Most of the S1P in tissues is believed to reside inside the cells, with little to no S1P in the interstitium. This assumption, however, mainly relies on the hypothesis that the concentration gradient between tissues, on the one hand, and blood and lymph, on the other hand, is
important for the maintenance of lymphocyte circulation. The lymphopenic phenotype of S1P receptor type 1 (S1PR1)-deficient mice indicates that lymphocytes sense S1P in the environment via S1PR1, which enables them to migrate along an S1P gradient (Matloubian et al., 2004). Lymphocytes enter lymph nodes from circulation with the guidance of chemokines via high endothelial venules (Forster et al., 1996; Forster et al., 1999). In circulation with high S1P concentration, S1PR1 is internalized due to phosphorylation by the G protein-coupled receptor kinase GRK2 among others (Arnon et al., 2011). In lymph nodes, S1PR1 is re-expressed on the surface of lymphocytes due to low S1P concentrations, which are maintained by S1P-lyase (Schwab et al., 2005). After re-expression of S1PR1 on the cell surface, lymphocytes are again able to follow an S1P gradient, which allows them to exit lymph nodes and to re-enter circulation (Lo, Xu, Proia, & Cyster, 2005). Such fine-tuning of S1P and S1P receptor function is highly dependent on different local and systemic S1P concentrations, and the determination of S1P concentrations in various fluids, cells and tissues under different conditions and disease states results in important molecular and mechanistic insights into regulatory pathways involving S1P and S1P receptor signalling.

The amount of S1P in tissues also varies in certain disease states, after pharmacological treatments and with genetic alterations in S1P-metabolizing enzymes (Mizraian et al., 2016; Sensken et al., 2010; Xuejing et al., 2019). Whereas increasing S1P concentrations in tissues were frequently reported after certain treatments or in S1P-lyase-deficient mice (Schwab et al., 2005; Vogel et al., 2009; Xuejing et al., 2019), decreasing S1P concentrations, particularly in tissues, are more challenging to detect and were rarely reported (Rieck et al., 2017; Sensken et al., 2010). The herein-reported improvement in low-level S1P detection in tissues may help identify the involvement of S1P metabolism and signalling in conditions that are currently undetected.

Quantification of S1P initially started with thin-layer chromatography (TLC) after radioactive labelling (Yatomi et al., 1995). A major breakthrough in the analytical methods was the use of o-phthalaldehyde, which was routinely used for the detection of peptides and amino acids (Butter, Koopmans, & Michel, 2005; Ruwisch, Schafer-Korting, & Kleuser, 2001). It reacts with primary amines, and this reaction produces a fluorophore that can be detected with high sensitivity. Similar strategies like the use of the amino-protecting group 9-fluorenylmethyl chloroformate or napthalene-2,3-dicarboxaldehyde further increased the stability and sensitivity of this HPLC method (Andreani & Graler, 2006; H. Huang, & Schuchman, 2009). A major disadvantage of all these methods was their limited specificity, which was a problem particularly with complex biological matrices like tissue extracts that contain a lot of unrelated amines and phosphates and produce many unspecified signals. The next major breakthrough in the analysis of S1P was LC coupled to triple-quadrupole MS (LC–MS/MS) (Schmidt, Schmidt, & Geisslinger, 2006). This method added another layer of specificity to TLC- and HPLC-based methods and has evolved as the standard analytical method for S1P quantification. Whereas the separation of sphingolipids is typically performed using reversed-phase HPLC, detection is dependent on MS. The reported sensitivities of LC–MS/MS methods were mainly dependent on the instrumentation used.

A study using the Agilent 6530 QTOF instrument reported a limit of quantification (LOQ) of 50 nM S1P (injection volume not specified) (Egom et al., 2017), whereas the use of the Sciex 4000 QTrap instrument provided an LOQ of 26 nM (Schmidt, Schmidt, & Geisslinger, 2006: 130 fmol on column; Xueijing et al., 2019: 52 fmol on column) (Schmidt, Schmidt, & Geisslinger, 2006; Xueijing et al., 2019) and the Sciex 3200 QTrap an LOQ of 6 nM (60 fmol on column) (Scherer, Schmitz, & Liebisch, 2009) or 12 nM (60 fmol on column) (Cutignano, Chiuminatto, Petruzzelli, Vella, & Fontana, 2010). All QTraps were operated in the multiple reaction monitoring (MRM) mode. The use of Xevo TQ-S micro (Waters, Eschborn, Germany) provided an LOQ of 1 nM (10 fmol on column) (Mizraian et al., 2016), and application of the TSQ Quantiva (Thermo Fischer Scientific, Waltham, MA, USA) resulted in an LOQ of 0.132 nM (0.528 fmol on column) (Saigusa et al., 2014).

The low abundance of S1P in cells and tissues requires very low detection and quantification limits. We therefore developed a highly sensitive LC–MS/MS method using the Sciex 2000 QTrap mass spectrometer to quantify S1P with an LOQ of 3 nM (30 fmol on column) in biological samples. This high sensitivity with basic instrumentation was reached using the linear ion trap function of the 2000 QTrap mass spectrometer to quantify S1P.

2 | MATERIALS AND METHODS

2.1 | Chemicals and mice

S1P was purchased from Sigma-Aldrich (Taufkirchen, Germany). S1P was dissolved at a concentration of 1 mM and diluted to a final concentration of 1 μM in methanol (MeOH). LC–MS-grade Chromasolv, Honeywell Riedel-de-Haën, Seelze, Germany). C17-Sph was purchased from Avanti Polar Lipids, Inc. (Otto Nordwald, Hamburg, Germany). All other chemicals and solvents were purchased from Roth (Karlsruhe, Germany) if not stated otherwise. C57BL/6 wild-type (WT) mice were purchased from the animal facility, Jena University Hospital, Jena, Germany. C17-Sph was purchased from the animal facility, Jena University Hospital, Jena, Germany. Sphingosine kinase 1 knockout (SphK1<sup>−/−</sup>) and sphingosine kinase 2 knockout (SphK2<sup>−/−</sup>) mice were kindly provided by Richard Proia (NIH, Bethesda, MD, USA). Animals were maintained under automatically controlled 12-h day–night cycles, temperature at 20–24°C and 50–60% humidity. Mice received a standard diet (Sniff, Soest, Germany) and water ad libitum. Mice were kept in groups of three to five. Cages were pre-sterilized and enriched with wood shavings and wood pulp.

2.2 | Sample preparation and lipid extraction

C57BL/6 WT, SphK1<sup>−/−</sup> and SphK2<sup>−/−</sup> mice were euthanized by inhalation of 5% isoflurane (Actavis, Munich, Germany). Mice were positioned on their back and disinfected with 70% ethanol (Carl Roth, Karlsruhe, Germany), and the organs were exposed by opening the abdomen. Mice were perfused through the left ventricle with phosphate-buffered saline (PBS) supplemented with 0.1% heparin (Biochrom, Berlin,
Germany). Heart and liver were harvested. Liver tissue (10–26 mg) was homogenized using a Stomacher 80 Micro Biomaster (Seward Limited, Worthing, UK). The tissue was transferred into a Stomacher 80 BA6046 bag (Seward Limited). Five millilitres of PBS and 10 μL of 30 μM C17-Sph as an internal standard were added and homogenized for 2 min. The heart tissue (21–24 mg) was homogenized by hand due to its rigidity using a pestle and mortar. It was transferred into a Stomacher 80 BA6040 micro bag, shock frozen in liquid nitrogen for 5 s and carefully crushed with the pestle in the mortar until it was powdered. The powder was resuspended in 1 mL of PBS, and 10 μL of the internal standard was added. The homogenate was transferred into a glass centrifuge tube (99449-16, Corning, Amsterdam, the Netherlands). The bag was rinsed once with 1 mL of MeOH, which was also transferred into the glass tube. All steps were performed on ice. Lipid extraction followed the Folch extraction method (Folch, Lees, & Sloane Stanley, 1957) under acidified conditions. Briefly, 300 μL of 6 M hydrochloric acid, 1 mL of MeOH (already added for heart samples) and 2 mL of chloroform were added to the homogenate. After vortexing for 10 min, the suspensions were centrifuged at 1900 rcf for 3 min. The lower organic phase was transferred to a new glass centrifuge tube, and the inorganic phase was extracted for a second time with 2 mL of additional chloroform as described earlier. The organic phases were combined and evaporated to dryness for 45 min at 60°C in the RVC 2-25 CD plus vacuum concentrator (Christ, Osterode am Harz, Germany; vacuum system by Vacuubrand, Wertheim, Germany).

2.3 | LC–MS/MS analysis

The extracts were reconstituted in 100 μL of MeOH/chloroform (4:1, v/v), and 10 μL was injected into the column. For spiking experiments, 25, 50 or 75 nM S1P was added to the reconstituted liver tissue extracts before injection. For the liver calibration curve, 1, 3, 6, 10, 30, 60 and 100 nM S1P were spiked to extracted liver samples. The HPLC system consisted of the L-7250 autosampler (Merck Hitachi, Darmstadt, Germany), the Merck peltier sample cooler for L-7250 (series 1100, Biorad, München, Germany) and the L-2300 column oven (Merck Hitachi), the G1322A degassing unit, the G1212A binary pump (Series 1100, Biorad, München, Germany) and the L-2300 column oven (Merck Hitachi) with the 60 × 2 mm MultoHigh 100 RP 18 column with 3-μm particle size (CS-Chromatographie Service, Langerwehe, Germany). The HPLC programme was as follows: 0 min 10% solvent B (MeOH), 90% solvent A (1% (v/v) formic acid in H2O), 0.1 min 100% solvent B, 15 min 100% solvent B, 15.1 min 10% solvent B, 20 min 10% solvent B, flow rate 0.3 mL/min. The HPLC system was coupled to a 2000 QTrap triple-quadrupole mass spectrometer (Sciex, Foster City, CA, USA) equipped with an ESI source operating in positive mode. The MS programme was divided into three separate parts. The first part started with the sample injection and was recorded in the MRM mode to measure the internal standard C17-Sph (m/z 286/268). After 4.5 min, S1P was measured in the enhanced product ion (EPI) mode for 2.1 min (m/z 380/264). The EPI mode started immediately after the MRM mode ended without an additional start signal. An optional recording in the MRM mode was added for an additional 8.4 min to detect more lipophilic compounds and started immediately after the EPI mode ended, again without an additional start signal. Column equilibration started again for 5 min with 10% solvent B. The parameters were optimized for S1P measurements in EPI and MRM (Table 1). Data were analysed using Analyst 1.6.2 (AB Sciex, Darmstadt, Germany).

2.4 | Validation of the analytical method

The method was validated for selectivity, accuracy and precision, linearity, matrix effect and recovery. The selectivity of the method was investigated by spiking the reconstituted liver tissue extracts from SphK1−/− or SphK2−/− mice of known S1P concentrations with 50 nM S1P to ensure that there were no interfering substances at the same retention time. Linearity was determined by spiking methanol or lipid extracts from liver tissue of SphK1−/− mice with S1P (0, 1, 3, 6, 10, 30, 60 and 100 nM). These standard curves were run and analysed on three consecutive days. The recovery rate was calculated based on the results derived from spiking experiments. Intra-day precision and accuracy were determined by consecutive analysis of the S1P content in quality control samples (mouse liver tissue samples spiked with 25, 50 or 75 nM S1P) five times. Inter-day precision and accuracy were investigated by the analysis of three individual runs with five replicates each of the S1P content in quality control samples at low (25 nM), middle (50 nM) and high (75 nM) levels. Precision was calculated as the percentage of relative standard deviation, and accuracy was expressed as the percentage of relative error. Matrix effect was

| Parameter | MRM | EPI |
|-----------|-----|-----|
| Curtain gas (CUR) | 40 | |
| Ion spray voltage (IS) | 5500 | |
| Temperature (TEM) | 350 | |
| Gas 1 (GS1) | 20 | |
| Gas 2 (GS2) | 40 | |
| Collisionally activated dissociation | Medium | |
| Declustering potential | 45 | |
| Entrance potential | 4.8 | |
| Cell entrance potential | 18 | |
| Collision energy | 19.5 | |
| Cell exit potential | 6.0 | |
| C2B | −400 | |
| Collision energy spread | 0 | |
| AF3 | 0.17 | |
| Exit barrier | −178.96 | |
| Q3 entry barrier | 10 V | |
| LIT fill time | 5000 ms | |
| Scan mode | Profile | |
| Scan rate | 250 Da/s | |

Note. EPI, enhanced product ion; LIT, linear ion trap; MRM, multiple reaction monitoring.
3 | RESULTS AND DISCUSSION

3.1 | Evaluation of the EPI scan mode for S1P quantification

S1P is a low-abundant sphingolipid signalling molecule that requires highly sensitive detection systems for quantification. Although most systems are suitable to quantify S1P in blood, plasma or serum, tissue extracts and lymph are more challenging and usually require sophisticated instrumentation. In an attempt to improve the sensitivity of our basic Sciex 2000 QTrap mass spectrometer for the detection of S1P, we started to optimize the instrument in the EPI mode for S1P. For the EPI scan, S1P is fragmented in the collision cell, and specific fragments are collected in the third quadrupole that acts as a linear ion trap. Because all captured fragments are detected collectively, higher sensitivity in S1P detection can be obtained. In contrast to other related compounds like FTY720-phosphate (2-amino-2-[2-(4-octylphenyl)ethyl]-1,3-propanediol, 1-(dihydrogen phosphate)) or Sph, the detection of S1P was significantly enhanced in EPI. The most relevant parameter for optimization besides the shared parameters of MRM and EPI modes was the EPI-specific value for Exit Barrier (Table 1). The optimization of this particular parameter resulted in a profound signal amplification that exceeded the sensitivity of the MRM scan mode (Figure 1a).

3.2 | Establishment of quantitative S1P measurements using the EPI scan mode

To use the optimized EPI scan mode to quantify S1P with increased sensitivity, the third quadrupole of the mass spectrometer was

![FIGURE 1](image-url) Sensitivity, selectivity and specificity of the EPI mode. (a) Chromatograms for the detection of increasing amounts of S1P in enhanced product ion (EPI) and multiple reaction monitoring mode recorded with the same 2000 QTrap instrument. The expected retention time of the signals is indicated by an arrow. (b) Retention times of the analyte S1P together with the internal standards C17-S1P and C17-Sph. (c) Liver extracts of C57BL/6 wild-type mice were spiked with 50 nM S1P. Peak intensity increased at the expected retention time for S1P in the EPI scan mode relative to the added amount of S1P.
operated as a linear ion trap with the maximal dwell time of 5000 ms. Due to the limitation that only one analyte could be measured in the EPI scan mode, C17-Sph was selected as an internal standard for this method. C17-Sph was sufficiently separated from S1P to be detected in the MRM scan mode before switching to the EPI scan mode. In contrast to C17-Sph, C17-S1P was not separated well enough to be measured by a different scan mode (Figure 1b).

3.3 | Evaluation of the specificity and selectivity of the EPI scan mode for S1P quantification

To determine the selectivity and specificity of the EPI scan mode for S1P, liver tissue of SphK1−/− mice was spiked with known concentrations of S1P and subsequently analysed. The peak areas of signals at the retention time of S1P increased as expected (Figure 1c). No additional unspecific signals were observed.

3.4 | Precision of quantitative S1P measurements with the EPI scan mode

Precision was determined as the percentage of coefficient of variation and was therefore calculated as 100 times the standard error divided by the mean. The resultant intra-day precision ranged between –3.8 and 6.3% depending on the concentration of the spiked S1P (Table 2). Inter-day precision was determined to be –13.8–3.3% (Table 2). The determination of C17-Sph as the internal standard was performed with the MRM scan mode before switching to the EPI scan mode for sensitive S1P measurements and was used for all calculations.

3.5 | Determination of linearity, LOD and LOQ for S1P measurements using the EPI scan mode

Linearity was determined by analysis of S1P standard curves (1, 3, 6, 10, 30, 60 and 100 nM), dissolved in pure MeOH and in lipid extracts from liver tissue of SphK1−/− mice, that were run on three consecutive days. The method was linear ($r^2 > 0.99$) in the range of 1–100 nM S1P (Figure 2). The LOD, defined as the required concentration of the analyte to produce a signal intensity of at least 3 standard deviations above the mean of the blank, was 1 nM S1P in pure MeOH and 3 nM S1P in lipid extracts from the liver tissue of SphK1−/− mice. The LOQ, defined as the required concentration of the analyte to produce a signal intensity of at least five times above the zero calibrator, was 3 and 10 nM S1P, respectively. In MRM mode, both limits were up to 30 times higher.

3.6 | Recovery of S1P from biological samples

To evaluate the recovery of S1P signals in biological samples, liver tissue of SphK1−/− mice was spiked with known concentrations of S1P, and the resulting signal was analysed with a background-free standard curve with known S1P concentrations dissolved in pure MeOH (Figure 2). The calculated recovery rates ranged between 44 and 55% starting from the LOQ of 10 nM S1P depending on the spiked S1P concentration (Table 3). The main reason for the loss of signal intensity in biological samples was a demonstrated matrix effect (Figure 2). The higher means of calculated recovery rates with S1P concentrations below the LOQ of 10 nM S1P can be explained by the higher standard deviation and increased spreading of the values and therefore do not represent exact measurements anymore (Table 3).

3.7 | Stability and carryover of S1P

To test the stability of S1P in sample preparations, liver tissues of SphK1−/− mice were spiked with 25 and 75 nM S1P and analysed immediately, 16 and 32 h after storage in the autosampler at 4°C and 7 days after storage at –80°C in the freezer. All signal intensities of stored samples were in the range of 97–117% compared to the immediate analysis, and no significant losses were observed (Table 4). Carryover of S1P was 57 ± 9% with 1 nM S1P and decreased to 16 ± 3% with 100 nM S1P (Table 5). The determination of very low S1P concentrations therefore required the inclusion of one to three blanks between samples.

3.8 | Method application for biological samples

To test the developed highly sensitive S1P quantitation method for application in biological samples with very low concentrations of S1P, liver and heart tissue lipid extracts from WT, SphK1−/− and SphK2−/− mice were analysed. S1P signals in liver extracts of SphK1−/− and SphK2−/− mice were twice as high as the LOQ, and S1P signals in liver extracts of WT mice were five times higher than the LOQ. The Extracts of hearts from SphK1−/− and SphK2−/− mice revealed S1P signals that were more than 10 times above the LOQ, and those from WT mice demonstrated S1P signals that were up to 19 times higher than the LOQ.

### Table 2: Precision and accuracy of the QTrap method for S1P quantification

| Test Parameter | Intra-day | Inter-day |
|----------------|-----------|-----------|
|                | Low      | Mid      | High     | Low      | Mid      | High     |
| Mean (nM)      | 26.6     | 52.5     | 72.2     | 25.8     | 47.3     | 64.7     |
| SD (nM)        | 2.7      | 6.7      | 9.9      | 0.9      | 3.8      | 6.7      |
| Precision (RSD, %) | 10.0   | 12.7     | 13.7     | 3.5      | 8.0      | 10.4     |
| Accuracy (RE, %) | 6.3    | 5.1      | –3.8     | 3.3      | –5.5     | –13.8    |

Note. Spiked S1P concentrations: low, 25 nM S1P; mid, 50 nM S1P; high, 75 nM S1P; n = 5 (intra-day); n = 11–15 (inter-day). RE, relative error; RSD, relative standard deviation; SD, standard deviation.
In knockout mice, S1P concentrations were about 50% of those in WT mice in both tissues (Figure 3). Liver tissue from SphK1−/− mice contained, in average, 14 nmol/kg (14 nM) S1P, SphK2−/− mice 15 nmol/kg (15 nM) S1P and WT mice 26 nmol/kg (26 nM) S1P. The detected mean concentrations in heart tissues were 61 nmol/kg (61 nM) S1P (SphK1−/− mice), 52 nmol/kg (52 nM) S1P (SphK2−/− mice) and 113 nmol/kg (113 nM) S1P (WT mice).

**FIGURE 2** Standard curve of S1P measured in the enhanced product ion (EPI) mode. Standard curves (0, 1, 3, 6, 10, 30, 60 and 100 nM S1P) were run using the EPI mode with and without biological background (mouse liver tissue). Mean ± SD is shown; n = 3

**TABLE 3** Recovery of S1P from mouse liver samples

| Spiked S1P (nM) | Recovered S1P (nM) | Expected S1P (nM) | Recovery (%) |
|----------------|--------------------|-------------------|--------------|
| 0              | 2.1 ± 0.3          | 3.1               | 103 ± 60     |
| LOD            | 3                  | 5.1               | 76 ± 27      |
|               | 6                  | 8.1               | 52 ± 12      |
| LOQ            | 10                 | 12.1              | 49 ± 6       |
|               | 30                 | 32.1              | 44 ± 18      |
|               | 60                 | 62.1              | 55 ± 10      |
|               | 100                | 102.1             | 46 ± 6       |

Note. Recovered S1P is shown as mean ± SD, n = 3; LOD, limit of detection; LOQ, limit of quantification; SD, standard deviation.

**TABLE 4** Stability of S1P for 16 and 32 h at 4°C in the autosampler and for 7 days at −80°C in the freezer

| Parameter | 4°C, low | 4°C, high | −80°C, low | −80°C, high |
|-----------|----------|-----------|------------|-------------|
|           | 0 h      | 16 h      | 32 h       | 0 d         | 7 d         | 0 d         | 7 d         |
| Mean      | 27       | 28        | 32         | 63          | 65          | 66          | 34          | 33          | 72          | 79          |
| SD        | 2        | 7         | 4          | 16          | 2           | 9           | 5           | 10          | 10          | 16          |
| Stability (%) | 105   | 117       | 102        | 105         | 97          | 110         |

Note. Spiked S1P concentrations: low, 25 nM S1P; high, 75 nM S1P; n = 3–4.

**TABLE 5** Carryover of S1P in biological samples

| Spiked S1P (nM) | Carryover (%), first blank | SD | Carry-over (%), 2nd blank | SD |
|----------------|---------------------------|----|---------------------------|----|
| 1              | 57                        | 8.5| 27.4                      | 5.0|
| 3              | 39                        | 5.1| 10.2                      | 0.9|
| 10             | 23                        | 4.9| 6.3                       | 2.3|
| 30             | 18                        | 2.5| 4.6                       | 0.5|
| 100            | 16                        | 3.3| 4.6                       | 0.6|

Note. n = 4.

3.9 Advantages and limitations of the applied QTrap method

Quantification of S1P in EPI mode provided considerably increased sensitivity compared to the commonly used MRM mode on the same instrument. The reason for this boost in sensitivity is not clear and may be due to specific hardware components and/or specific
Highly sensitive and specific quantification of S1P has become important since the past decade because of the broad range of S1P concentrations in different cell types, tissues and body fluids. We developed a validated detection method for S1P with high sensitivity using the 2000 QTrap mass spectrometer that provides the advantage of the linear ion trap function. With an LOD of 1 nM S1P (10 fmol on column) it is one of the most sensitive S1P detection methods reported. The method includes quick and simple lipid extraction and requires only little amounts of tissue or cells. Accurate quantification of S1P was possible in liver tissues containing as little as 14 nmol/kg S1P.

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**CONFLICT OF INTEREST**

The authors declare no potential conflict of interest.

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