Interdisciplinary Social Research
Volume 2, Issue 2
ISSN: 3067-7238

SCHOLARLY SUMMIT
THE ACADEMIC PUBLISHERS

LC–MS/MS Steroid Profiling in Maternal and Neonatal Health: Analytical Advances and Clinical Implications

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Abstract

Steroids have crucial functions in maternal physiological changes and in neonatal transition at birth, but the analytical difficulties in quantifying these hormones are due to low concentrations, structural similarities and complexity of biological samples. Recent advances in simultaneous determination of four important steroid hormones (cortisol, progesterone, estradiol and androgens) in maternal and neonatal samples using LC–MS/MS technique and analytical challenges and future perspectives are presented. A comprehensive literature search was performed, especially regarding the LC–MS/MS methods, chromatographic optimization, multiple reaction monitoring (MRM), and the sample preparation process of serum, plasma, cord blood and dried blood spots (DBS). Advancements in LC–MS/MS technology, have led to sensitive, high selectivity and high throughput profiling of steroids at low concentrations. Optimized chromatography, MRM transitions and isotope dilution provide increased quantitative reliability. However, there are some drawbacks such as matrix effect, suppression of ions, isobaric interference, small sample volume, and lack of standardization. In some clinical situations, better steroid profile may be helpful for detecting complications of pregnancy before they occur and may even detect neonatal endocrine disorders which would lead to better neonatal and maternal care. The continued application of LC–MS/MS in clinical work also suggests the use of precise determination of hormones in clinical applications such as precision medicine, neonatal screening, and in healthcare systems of limited resources, where precise evaluation of the hormones could help to improve the early intervention, diagnosis and evidence-based care of mothers and children.

Keywords: LC–MS/MS; Steroid hormones; Cortisol; Progesterone; Estradiol; Maternal; Neonatal; Steroidomics.

1. Introduction

Steroid hormones are essential endocrine regulators in almost all physiological systems, such as developmental programming, reproduction, stress response, immune modulation, and metabolism. Steroid hormones like cortisol, progesterone, estradiol and androgens are synthesized in the adrenal glands, gonads, and placenta through tightly regulated enzymatic pathways and form an interconnected biochemical network that regulates systemic homeostasis (Mason et al., 2020; Pignatti et al., 2023). However, steroid biosynthesis is a tightly coupled pathway, so changes in one hormone usually impact several metabolites downstream, and, therefore, single analyte analysis is not enough to fully assess the endocrine status of a system (Andrew & Homer, 2020).

Pregnancy is one of the most dynamic endocrine changes in human physiology with ongoing endocrine adaptation in the maternal–placental–fetal unit. Steroid hormones control important functions like implantation, formation of the placenta, immune tolerance and fetal growth. Cortisol plays a role in fetal organ maturation, progesterone in maintaining uterine quiescence, and estrogens in controlling uteroplacental blood flow and fetal development (Esposito et al., 2025). Likewise, during the neonatal period, steroid hormones play a vital role in adrenal activation, metabolic maturation, stress response, and neurodevelopmental adaptation (Olthof et al., 2025). Significant clinical consequences associated with dysregulation of steroid pathways are found in preeclampsia, gestational diabetes mellitus, fetal growth restriction, congenital adrenal hyperplasia, and preterm birth (Fowden et al., 2022).

Although clinically significant, quantification of steroid hormones in maternal and neonatal biological samples is analytically difficult. The composition and complexity of popular matrices such as serum, plasma, urine, saliva, amniotic fluid, cord blood and dried blood spots (DBS) vary significantly and may contain interfering endogenous compounds (Price et al., 2023; Struck-Lewicka et al.,

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Received: 6-May-2026 Revised: 16-May-2026 Accepted: 3-June-2026

Interdisciplinary Social Research
Volume 2, Issue 2
ISSN: 3067-7238

2022). In addition, steroid hormones are present in minimal quantities in the biological system and there is also a similarity in the structure of the steroids, which leads to ion suppression and matrix effects and analytical cross reactivity (Karashima & Osaka, 2022). The problems are especially severe for neonatal studies where sample volumes are small and physiological changes occur quickly after birth.

Traditionally, immunoassays have been employed to determine the levels of steroid hormones; Despite their many advantages, their use is hindered by a lack of specificity, antibody cross-reactivity, and the inability to measure more than one hormone at a time, which can result in false results in complex biological samples (Dinkelbach et al., 2025). The restrictions have posed a great need for more accurate and multiplex analytical platforms.

The liquid chromatography–tandem mass spectrometry (LC-MS/MS) method has become the gold standard for steroid hormone analysis because of its high sensitivity, selectivity, and ability to quantify multiple analytes simultaneously (Keevil, 2013; Soldin & Soldin, 2009; Taylor et al., 2015). The analytical performance of recent developments of Ultra-High-Performance Liquid Chromatography (UHPLC) systems, UHPLC triple quadrupole instrumentation, isotope-labelled internal standards, and multiple reaction monitoring (MRM) has improved significantly (Chai et al., 2023). Steroidomics can also provide total profiling of steroid pathways from small sample sizes that can be valuable in neonatal research, in parallel (Storbeck & O'Reilly, 2023).

Despite the advancement of technology, there are still challenges that need to be addressed, such as the absence of standardization between laboratories, inconsistency in sample preparation, analytical bias or dependence on the sample matrix, and poor harmonization of reference methods (Tate et al., 2014; Thienpont et al., 2017). More specifically, a comprehensive overview specifically addressing simultaneous steroid quantification in maternal and neonatal matrices using LC-MS/MS, incorporating analytical approaches and clinical relevance, is currently missing.

The purpose of this review is to critically evaluate recent developments in LC-MS/MS methodology for the simultaneous determination of steroid hormones in maternal and neonatal biological matrices, with a focus on analytical developments, method challenges and translation. The hypothesis is that the multiplex LC-MS/MS steroid profiling will yield better analytical accuracy, lower sample usage and better clinical interpretation than the traditional single analyte approach.

2. Methodology

2.1. Review Design

The present study was carried out as a structured narrative review to integrate the existing information regarding the quantification of steroid hormones in maternal and neonatal biological matrices on the basis of LC–MS/MS analysis. This review will address analytical progress, methodological validation strategies and clinical applications of steroidomics in perinatal endocrinology. A narrative review is different from a systematic review, which strictly adheres to protocols for quantitative synthesis, and can integrate heterogeneous evidence from analytical chemistry, clinical endocrinology, and translational research to gain a broader conceptual understanding of steroid hormone measurement in complex biological systems (Edwards, 2022; Green et al., 2006).

2.2. Literature Search Strategy

A systematic literature search was conducted to locate relevant peer-reviewed literature. The electronic databases PubMed/MEDLINE, Scopus, Web of Science and Embase were systematically searched. The search strategy included the use of Medical Subject Headings (MeSH) as well as free-text keywords, including: "LC-MS/MS", "liquid chromatography tandem mass spectrometry", "steroid hormones", "steroidomics", "cortisol", "progesterone", "estradiol", "maternal serum", "pregnancy", "neonatal", "cord blood", "dried blood spots", and "biological matrices". Boolean operators (AND, OR) were used to refine searches. The literature included was limited to publications in English and from 2015 to 2025, with special emphasis on research published since 2020 to make sure the most recent analytical and technological developments were included.

2.3. Study Selection

All the studies were chosen according to their relevance in the context of steroid hormone analysis in maternal and neonatal matrices by LC-MS/MS. Only original research articles, method development studies, analytical validation reports and high-quality reviews reporting quantitative steroid measurements in human samples were included. After initial title and abstract screening, full-text screening was performed to determine the methodological relevance.

2.4. Eligibility Criteria

The studies included fulfilled criteria of analysis using LC-MS/MS or hybrid mass spectrometry; analysis of biological matrix of neonatal or maternal origin (serum, plasma, urine, saliva, DBS, cord blood, amniotic fluid); and reporting single or multiplex steroid measurements, including analytical performance data (e.g., sensitivity, specificity, linearity, LOD, LOQ, recovery).

Only studies that were in English, solely immunoassays without an LC-MS/MS comparison, were animal-only studies with no relevance to humans, or provided insufficient methodological detail were excluded. In addition, editorials, commentaries, and conference abstracts were excluded to ensure scientific rigor.

2.5. Data Extraction and Data Synthesis

The data was extracted using a standardized data framework, encompassing all of the steroid analytes, biological matrices, sample preparation, chromatography, ionization, and mass spectrometric parameters. The other analytical validation metrics, accuracy, precision, recovery, matrix effects, Limit of Detection (LOD) and Limit of Quantification (LOQ) were also obtained.

A qualitative narrative synthesis approach was used to integrate findings. The studies were broken down into themes: (i) single and multiplex steroid quantification using LC-MS/MS, (ii) matrix-specific challenges in the analysis of mothers and their neonates, (iii) advances in sample preparation and instrumentation, and (iv) clinical application in pregnancy and neonatal endocrinology. This method allowed the incorporation of various pieces of analytic and clinical information in a logical, coherent structure.

2.6. Conceptual Framework

A conceptual framework was drafted to show how biological matrices relate to analytical challenges, LC-MS/MS workflows, and clinical applications. It demonstrates the complexity of steroid biology and matrix variability, and its application of cutting-edge LC-MS/MS techniques to better interpret steroidomics and to facilitate the assessment of maternal and neonatal endocrine status (Figure 1).

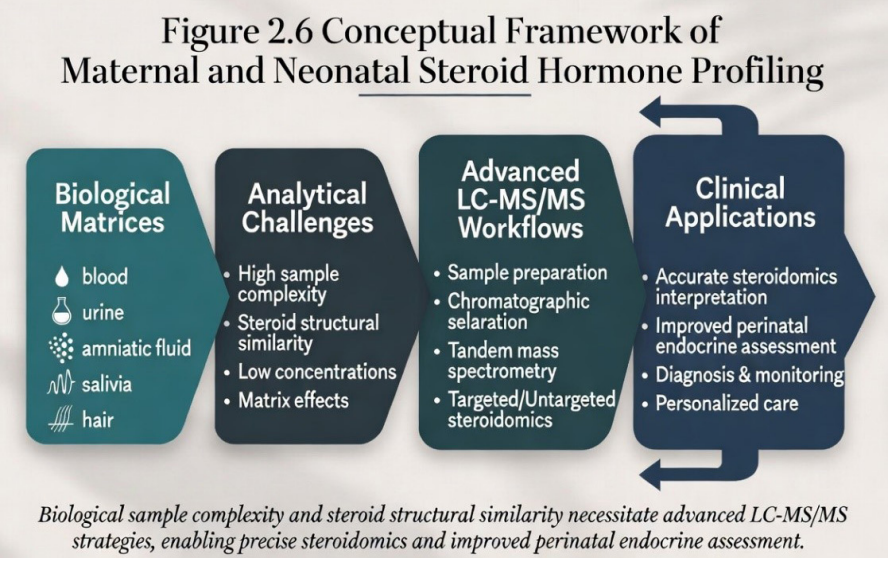

Figure 1. LC–MS/MS steroid analysis framework

3. Steroid Hormones of Interest in Maternal and Neonatal Systems

Steroid hormones are cholesterol-based endocrines that play an important role in metabolism, immune regulation, stress response, reproduction and fetal development. Steroidogenesis is a coordinated process between maternal adrenal and gonadal steroid production, enzymatic conversion in the placenta, and fetal adrenal steroid production in the maternal–placental–fetal unit. This integrated system ensures pregnancy, fetal growth, and adaptation of the newborn. However, because of their good structural similarity, LC-MS/MS is a preferred method over immunoassays, which are more prone to high cross-reactivity and less sensitive (Chai et al., 2023; Krasowski et al., 2014; Taylor et al., 2015).

The most important glucocorticoid hormone of the

Interdisciplinary Social Research
Volume 2, Issue 2
ISSN: 3067-7238

hypothalamic–pituitary–adrenal axis is cortisol. The level of maternal cortisol gradually rises during gestation because of the production of corticotropin-releasing hormone by the placenta; reported maternal–fetal gradients are of the order of 2–3 fold. This height facilitates fetal lung maturation, metabolic programming and stress preparation. Cortisol plays an important role in maintaining postnatal stability, glucose homeostasis and cardiovascular stability in neonates. There is an association between dysregulation and preterm birth and growth restriction. However, the low concentrations of these hormones in neonates can be hard to quantify, especially when levels of structurally similar corticosteroids are present, necessitating highly selective LC-MS/MS using optimized MRM transitions (Enver et al., 2022; Olthof et al., 2025).

The function of progesterone is to maintain pregnancy, which is produced in the first few weeks by the corpus luteum and later by the placenta. It keeps the uterus in a non-contractile state and tolerates immune cells. The concentrations rise regularly during gestation and are about 10–20-fold higher in late gestation than in early gestation. Neonatal levels are mostly a reflection of the maternal transfer and the peripartal endocrine transition. Changes are linked with preterm labor and miscarriage. The analytical measurement is complicated by the presence of isobaric steroids, which will need chromatographic separation and isotope-labelled internal standards for accurate quantification.

Estradiol (17β-estradiol) is the most active estrogen, which is mostly synthesized through fetoplacental steroidogenesis. It influences uteroplacental perfusion, vessel remodeling, and fetal growth. Estradiol levels increase exponentially during pregnancy, increasing more than 100-fold from early to late gestation, and a rapid fall occurs in the postpartum period. High-sensitivity LC-MS/MS platforms are required due to the low physiological concentrations and interferences from isobaric estrogens (Johänning et al., 2015; Wang & Mesaros, 2025).

The steroidomic network is complemented by other steroids that include dehydroepiandrosterone sulfate (DHEA-S), testosterone, corticosterone, and aldosterone. These compounds have a large dynamic range of several orders of magnitude and a similar fragmentation pattern that makes the simultaneous detection difficult. The reliable multi-analyte quantification can be achieved thanks to multiplex LC-MS/MS and isotope-labelled internal standards and MRM.

Steroid hormones are generally not biomarkers, but a system of hormones. Steroidomics based on LC-MS/MS can be performed in a single profile with high analytical fidelity, thereby enhancing the understanding of the maternal–neonatal endocrine adaptation and facilitating clinically relevant interpretation (Table 1).

Table 1. Steroid Hormones and Their LC-MS/MS Analytical Relevance in Maternal and Neonatal Samples

Steroid Hormone	**Biological Role**	**Maternal Relevance**	**Neonatal Relevance**	**Analytical Challenges (LC-MS/MS)**	**Clinical Significance**
Cortisol	Stress response, HPA axis	Pregnancy adaptation	Neonatal stress response	Low levels, ion suppression	Preterm birth, stress disorders
Progesterone	Pregnancy maintenance	Uterine stability	Placental transfer marker	Isobaric steroids	Preterm labor risk
Estradiol	Vascular & fetal growth	Placental hormone	Postnatal decline	Low concentration, interference	Preeclampsia, growth restriction

4. LC-MS/MS Analytical Methods for Steroid Quantification

Liquid chromatography–tandem mass spectrometry (LC-MS/MS) is the gold standard method for quantification of steroid hormones because of its high sensitivity, high selectivity, and its ability to detect multiple analytes simultaneously. Immunoassays have been hampered by antibody cross-reactivity, lack of specificity for structurally similar steroids and are therefore not well suited to complex maternal and neonatal biological matrices; LC-MS/MS offers direct molecular identification by mass-to-charge ratio and characteristic fragmentation pattern, which is highly suitable for complex matrices (Piombarolo, 2026; Wang & Mesaros, 2025) (Figure 2).

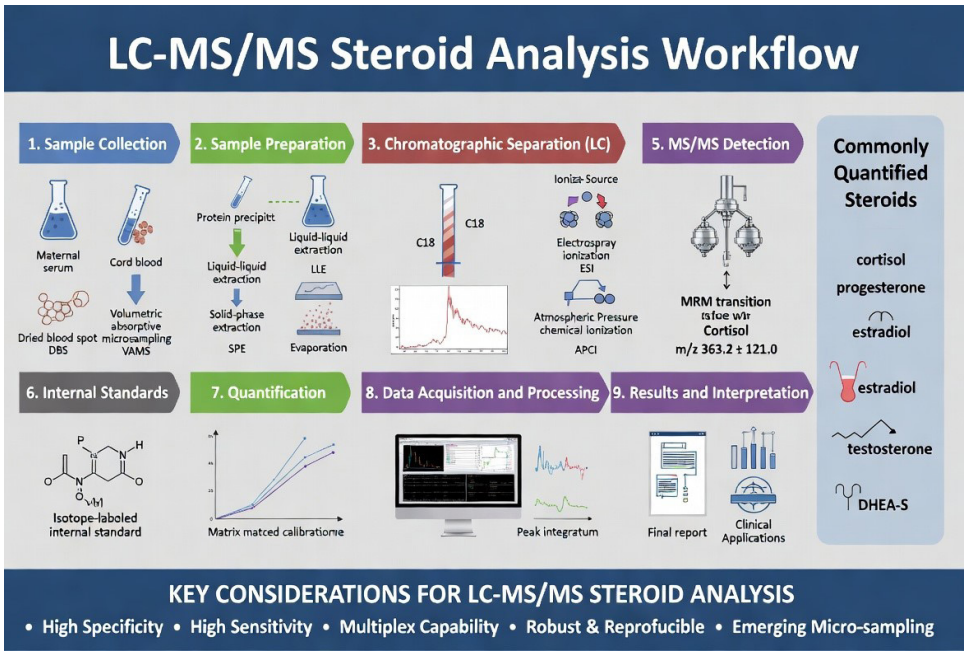

Figure 2. LC-MS/MS Workflow for Steroid Hormone Quantification

Steroid analysis relies heavily on chromatographic separation, which separates structurally similar and isobaric compounds before they can be detected in the mass. The reversed-phase liquid chromatography on C18 columns is the most popular method used because of the high affinity for hydrophobic steroid molecules. In more sophisticated uses, the phenyl or biphenyl columns provide a better separation of closely related steroid isomers. Gradient elution systems provide efficient separation in a relatively short time using aqueous buffers and organic solvents like methanol or acetonitrile. Optimize chromatography to minimize matrix effects and ion suppression in complex matrices, especially cord blood and plasma.

The sensitivity of the detection is related to the ionization. Electrospray ionization (ESI) is commonly used for steroid analysis particularly polar derivatives and gives stable ions at low pressure. However, for non-polar or unmodified steroids, atmospheric pressure chemical ionization (APCI) may provide more efficient ionization and less matrix interference. It is dependent on the sample complexity, the configuration of the instrument, and the polarity of the analyte.

The primary means of detection are multiple reaction monitoring (MRM) on triple quadrupole mass spectrometers, which enables highly selective and sensitive quantification of multiple steroid analytes in a single analytical run (Fu et al., 2016; Yu et al., 2024). In this mode, a group of precursor ions is selected for fragmentation and then the fragment ions are monitored in a predetermined sequence of product ions, which can be very analytically specific even in complex biological samples. However, they are stable isotope labelled internal standards which must be used to correct for extraction efficiency, ionization and instrument response for accurate quantification. The calibration curves are prepared using matrix-matched standards and are linear over the range of physiological concentrations usually in the picograms to nanograms range. In recent years, high-resolution mass spectrometry (HRMS) technologies, such as quadrupole time-of-flight (QTOF) and Orbitrap, have broadened the applications of steroidomic and improved mass accuracy and full-scan spectra acquisition. These provide facilities for the analysis of historical data and improved isobaric separation. However, for routine quantitative steroid analysis, the triple quadrupole LC-MS/MS system is the most popular due to the enhanced sensitivity, robustness and reproducibility.

The performance of the analytical method is influenced by many factors such as sample preparation, which can be performed in different ways, all of which are aimed at eliminating matrix interferences and enhancing analyte recovery (Table 2). Neonatal studies often rely on emerging micro-sampling technologies, like DBS and volumetric absorptive micro sampling, because of limited sample volume availability.

Interdisciplinary Social Research
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ISSN: 3067-7238

Table 2. Comparison of Analytical Platforms for Steroid Hormone Quantification

Feature	Immunoassays	LC-MS/MS	HRMS
Specificity	Low	High	Very high
Sensitivity	Moderate	High	High
Multiplexing	Limited	Excellent	Excellent
Cross-reactivity	High	Minimal	Minimal
Cost	Low	Moderate	High
Clinical use	Routine	Reference method	Research

Overall, LC-MS/MS combines optimized chromatography, selective ionization and highly specific detection strategies, all of which can be used to reliably quantify steroids in maternal and neonatal matrices, and form the analytical backbone of modern steroidomics research (Grebe & Singh, 2011; Taylor et al., 2015).

5. Sample Preparation and Matrix Considerations

The preparation of samples is one of the major factors that will affect the performance of the analytical method for quantification of steroid hormones using liquid chromatography mass spectrometry (LC-MS/MS). The compositional differences among biological matrices such as serum, plasma, urine, saliva, cord blood and DBS are a challenge in maternal and neonatal studies due to varying protein contents, lipid composition, and endogenous interfering substances. This complexity demands a careful optimization and preparation procedure for each matrix to ensure reliable steroid profiling (Ferreira et al., 2022; Jäger et al., 2023).

Protein precipitation (PPT) is one of the most common traditional methods, as it is simple and has a high throughput, but it is generally not very selective and can also lead to high matrix effects caused by phospholipids and endogenous compounds. An alternative technique is liquid–liquid extraction (LLE), which involves using non-polar steroid compounds (such as progesterone and cortisol) to enhance enrichment of analytes and produce cleaner extracts. Solid phase extraction (SPE) is considered the gold standard technique in the field of high precision steroid analysis, since it allows the most selective and reproducible adsorption of analytes on the functionalized sorbent phases, and the exclusion of interfering matrix components (Table 3).

Table 3. Sample Preparation Techniques

Method	Advantage	Limitation	Use Case
PPT	Fast	Poor clean-up	Routine screening
LLE	Good recovery	Time-consuming	Steroid extraction
SPE	High purity	Costly	Clinical LC-MS/MS
DBS/VAMS	Minimal volume	Hematocrit bias	Neonatal studies

One of the analytical constraints in neonatal applications is sample volume. The use of techniques like DBS, volumetric absorptive microsampling (VAMS), and non-invasive matrices such as saliva has become increasingly relevant, enabling less invasive steroid monitoring in pediatric and neonatal LC-MS/MS studies (Dahl et al., 2024; Resztak et al., 2025). The use of techniques like DBS and volumetric absorptive microsampling (VAMS) has become more and more relevant, which allows for less invasiveness and lower blood volume of samples (Ponzetto et al., 2024). These microsampling techniques, however, present extra variability because of hematocrit effects, extraction efficiencies, and spot-to-spot heterogeneity, and need to be optimized and validated carefully.

The ion suppression or enhancement in ESI is one of the biggest analytical challenges in the quantification of steroids by LC-MS/MS. These effects are caused by the presence of co-eluting endogenous compounds such as phospholipids, salts, and proteins, which affect the efficiency of and/or quantitative accuracy of the ionization. The effects can be reduced by careful sample clean-up and chromatographic optimization procedures. The evaluation and control of matrix-induced variations are usually realized by post-column infusion experiments and matrix

factor evaluations during method development.

The use of stable isotope labelled internal standards is crucial for the correction of matrix effects, extraction and instrumental variations. These structurally similar analogues co-elute with target analytes and compensate for analytical fluctuations to improve the accuracy and precision of quantifications in a wide range of biological samples. Furthermore, matrix-matched calibration curves are regularly employed to correct for any residual matrix difference to obtain linearity in the physiological concentration range.

Another important group of factors that should be monitored for steroid stability is pre-analytical factors, including collection, storage, hemolysis, and freeze-thawing. Steroid hormones are usually stable at frozen temperatures, but will change to other products if stored at sub-optimal temperatures or if repeatedly frozen and thawed, potentially affecting analytical results.

Finally, an efficient sample preparation and matrix management solution is essential for accurate and precise determination of steroids by LC-MS/MS. The factors are highly relevant in the area of maternal and neonatal research, where small sample volumes are coupled with high biological variability and require highly sensitive, selective and reproducible analytical workflows.

6. Method Validation Considerations

The validation of the method is also an indispensable aspect of steroids LC-MS/MS analysis, which is a reliable, reproducible and appropriate analytical procedure that will be applied in clinical and research uses. The importance of validation is particularly relevant for maternal and neonatal steroid profiling, due to the low concentration of the steroids in bio-matrices, the complex nature of the biological matrix, and the similarity in structure of the steroids in the profile. The US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have published bioanalytical validation guidelines applicable to all global agencies and set a minimum performance standard of quantitative LC-MS/MS methods (Aubry, 2011; Côté et al., 2009).

The most important validation parameters are selectivity and specificity, which guarantee the detection of target steroids without interference from endogenous and structurally similar metabolites. This is especially relevant to the field of steroidomics, where isobaric compounds or similar fragmentation patterns can lead to false positives or quantifications. Although LC-MS/MS can be utilized to increase selectivity, because of the nature of LC-MS/MS, it must still be validated by examining significant interference at the peak of the analyte for blank matrices.

Accuracy and precision are important methods performance measures. Precision indicates the reproducibility of the measured concentrations under the same conditions, while accuracy indicates the agreement of the measured concentrations with the true concentrations. These parameters are tested in low, medium and high concentration QC. The analytical result deviation is typically set at a range of ±15% (+/-20% at the lowest level of quantification) and is reproducible across analytical runs. Intra-day and inter-day precision testing should be conducted to confirm the method's stability and robustness through time.

The linearity of the response is determined by the preparation of calibration curves from matrix-matched standards, resulting in a detector that has a proportionate response in the physiological concentration ranges. The calibration range for steroid assays can be quite broad, depending on the amount of the steroid being measured, and nanogram to picogram levels of steroids are often used in steroid analysis. A weighted regression model is often used to improve the accuracy of the readings in the lower concentration ranges, where there is more variation.

Sensitivity is expressed as the limit of detection (LOD) and limit of quantification (LOQ), particularly in the neonatal applications where the steroid concentration is low. High-sensitivity LC-MS/MS systems are needed to assure detection without loss of specificity (Aubry, 2011). Recovery and extraction efficiency are also assessed to assess the percentage of analyte recovered from the biological matrix following sample preparation to ensure consistency across serum, plasma, cord blood and dried blood spot samples.

Matrix effects are considered one of the most complex analytical problems encountered in LC-MS/MS Steroid Quantitation. Endogenous components, such as phospholipids and salts, can be co-eluted and lead to suppression or enhancement of signals (Côté et al., 2009; Piombarolo, 2026). The effects are assessed by post-extraction addition experiments and matrix factor calculations. These differences can be overcome by the use of stable isotope labelled internal standards, thus improving the accuracy and reproducibility.

Stability testing confirms the stability of the steroid hormones under a variety of conditions, including short-term storage, long-term freezing, freeze-thaw and post-preparative handling. This is especially crucial in

Interdisciplinary Social Research
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ISSN: 3067-7238

multicenter studies and delayed sample analysis situations. Other challenges include limited sample size and biological variability in maternal and neonatal studies that require optimized validation methods.

Overall, method validation was used to prove that the steroid quantification by LC-MS/MS can meet international regulatory standards and provide quality data for clinical and translational applications in maternal and neonatal endocrinology.

7. Advances in LC-MS/MS Method Development

The sensitivity, specificity and speed of steroid hormone quantification in maternal and neonatal biological samples have significantly improved in recent years with the development of liquid chromatography–tandem mass spectrometry (LC-MS/MS). The technologies are mainly related to the clinical necessity for performing a complete steroid profile, the quantity of samples needed, and the need for rapid analytical turnaround times in the field of perinatal endocrinology. The paradigm shift is from single analyte assays to multiplex steroid quantification (steroidomics) enabling the quantification of multiple hormones in a single analytical run. This approach will not only conserve valuable neonatal samples, but also provide a more complete picture of endocrine regulation during pregnancy and early life (Jäger et al., 2023).

One of the most significant advances that occurred is the development of multi-steroid panels based on MRM. The optimized methods enable the measurement of all four classes of steroid hormones (glucocorticoids, progestogens, estrogens and androgens) in a more efficient way. When coupled to ultra-high performance liquid chromatography (UHPLC), the systems will enable highly resolving separation of structurally similar isomers of steroids in a much-reduced analysis time. Now, more samples can be processed without sacrificing analytical accuracy in clinical laboratories.

Another important aspect that impacts the performance of LC-MS/MS is sample preparation. Automated solid-phase extraction (SPE) systems improved operator-independent variability and provided greater reproducibility. The use of online extraction methods that are directly linked to LC-MS/MS systems further decreases manual handling and enhances analytical consistency (Badawy et al., 2022). However, blood volume is very small for studies in neonates, and as a result, there has been a trend toward the increasing importance of microsampling techniques, such as DBS and volumetric absorptive microsampling (VAMS). These methods involve minimal invasive sampling and can be applied with an acceptable analytical sensitivity, but a few aspects have to be optimized, such as hematocrit variation, extraction efficiency, etc.

There have also been instruments developed that have broadened the field of analysis. The high-resolution mass spectrometry (HRMS) platform, such as a quadrupole time-of-flight (QTOF) mass spectrometer, enables the separation of isobaric steroid compounds with high accuracy and mass resolution. While the routine quantification can be done more readily with the triple quadrupole instruments, the high sensitivity and robustness of these instruments make HRMS systems more and more relevant for untargeted steroidomic profiling and biomarker discovery.

A significant improvement has been the use of the internal stable isotope labelled standard. Co-elutes with target analytes and corrects for matrix effects, extraction losses and ionization variation to offer analytical accuracy and reproducibility across a wide range of matrices, including serum, plasma and cord blood.

Methodological improvement clinically has resulted in the LC-MS/MS application in the field of maternal and neonatal endocrinology. Steroid profiling is now widely used in pregnancy management, the early detection of pregnancy complications like preeclampsia and gestational diabetes, and the diagnosis of endocrine diseases in the newborn. Longitudinal steroidomics are also contributing to the identification of biomarkers and to understanding fetal programming and developmental physiology.

Finally, the steroid hormone analysis has moved from a one- to a higher throughput multiplex steroidomic platform, enabling clinical diagnostic and maternal and neonatal health translation studies.

8. Challenges, Limitations and Future Perspectives

LC/MS/MS has come a long way in the field of steroid hormone analysis, but a few challenges remain for applications in maternal and neonatal analysis. Another source of analytical variation in complex biological samples, such as serum, plasma or cord blood, is the matrix effect which refers to suppression or enhancement of the ion response due to endogenous compounds. Furthermore, the structural similarity of the steroids causes the additional isobaric and isomeric interference, rendering the complete chromatographic resolution even more challenging, even with MRM-based detection. In the neonatal studies, low levels of the hormones in the sample and the accelerated metabolism of hormones in early postnatal period makes

quantification difficult. In terms of clinical use, high instrument costs, infrastructure needs, and the demand for specialized expertise hinder broad clinical use, especially in resource-limited settings. These restrictions can lead to differences in access to second opinions for advanced tests, and to later diagnosis of pregnancy complications and neonatal disorders. Furthermore, the lack of standardized sample preparation, calibration and reporting procedures adds to the inter-laboratory variation and difficulty in comparing data.

Steroid profiling done using LC-MS/MS may be beneficial in early diagnosis of pregnancy complications and for the stratification and prompt clinical intervention in pregnancy conditions such as preeclampsia, gestational diabetes and preterm birth. Introducing the use of neonatal screening applications helps to detect congenital endocrine disorders in a timely manner and thereby achieve better outcomes and timely intervention. Additionally, maternal health can be improved by better monitoring of steroid changes throughout pregnancy for more accurate clinical decisions. In low-resource settings, however, access is a challenge because of limited infrastructure and cost, and this will need a more streamlined and scalable diagnostic approach to achieve parity in healthcare. Furthermore, in clinical practice, steroid profiling helps in achieving personalized medicine by assessing the individual hormonal status in the care of the mother and her newborn.

Future developments of larger multi-hormone profiling with lower sample volumes and higher automation levels of high-quantity steroidomics platforms are expected. In obstetrics and neonatology, emerging miniaturized and portable LC-MS/MS technologies could contribute to the point-of-care diagnostic accessibility and to the improvement of maternal–neonatal healthcare. The incorporation of AI and machine learning is expected to facilitate the analysis of intricate steroid patterns and facilitate earlier detection of pregnancy complications and conditions in newborns. Moreover, harmonization of the analytical processes and development of reference ranges will be essential for wider clinical translation and diagnostic equity. Overall, emerging technologies and techniques will continue to benefit the clinical, translational, and public health applications of LC-MS/MS in the study of maternal and neonatal endocrinology over the upcoming several years.

9. Conclusion

LC-MS/MS is the analytical platform of choice for steroid quantitative analysis in maternal and neonatal matrices. Compared to traditional immunoassays, the multiplexing and sensitivity of the method are better, and the specificity is superior. It incorporates optimized chromatographic separation, MRM and isotope-labeled internal standards for the simultaneous measurement of structurally related steroids over a broad concentration range. New advances in steroidomics have recently made it possible to profile the endocrine pathways involved in pregnancy and neonatal adaptation in detail. However, issues such as isobaric interference, matrix effects, a limited number of samples for neonates, and a lack of standardization have yet to be addressed for full clinical use. The future of high-resolution mass spectrometry will be characterized by continuous innovations in sample preparation, automated sample preparation, micro-sampling, and data analysis software. This should improve robustness and scalability. LC-MS/MS is a powerful translational tool that has significant potential to enhance the accuracy of diagnosis, risk stratification and clinical care of maternal and neonatal endocrine conditions if further harmonization and validation efforts are realized.

10. Declaration and Statement

Acknowledgements: The author would like to thank all individuals and institutions who provided support, guidance, or assistance in the preparation of this work, but do not meet authorship criteria.

Funding Statement: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions: The author is solely responsible for each contribution made to this research.

Conflicts of Interest: The author declares no competing interests or conflicts that could have influenced the content of this work.

Data Sharing Statement: The data supporting the findings of this study are included within the manuscript. No additional raw datasets were generated or analyzed beyond what is presented. Due to privacy considerations and the use of published data from third-party studies, individual patient-level data cannot be publicly deposited. Researchers interested in accessing specific study datasets should refer to the original publications cited in this review for data access details.

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Volume 2, Issue 2
ISSN: 3067-7238

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Interdisciplinary Social Research
Volume 2, Issue 2
ISSN: 3067-7238

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