



Dipharma's Playbook on Bile Acid Derivatives: Turning Complex Scaffolds into GMP Reality

APIs

for Generic
Market

CDMO

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The discovery of bile acid derivatives by Adolph Strecker goes back to 1848. Fundamental insights into bile acid structure and oxidation chemistry were later provided by Heinrich Otto Wieland, whose contributions were recognized with the Nobel Prize in Chemistry in 1927. In the same year, Masato Shoda first isolated ursodeoxycholic acid (UDCA) from bear bile. For decades, bile acids have been widely considered to be 'metabolic soaps' assisting the digestion and absorption of (dietary) fats in the small intestine, but more recently they have emerged as pharmacologically active molecules across a wide spectrum of diseases, from cholestatic liver conditions to rare metabolic disorders and neurodegeneration. Among them, **ursodeoxycholic acid (UDCA)** stands out as the pioneer compound, having received regulatory approval decades ago for the treatment of **primary biliary cholangitis (PBC)**.

Following its regulatory approval in the United States (ursodiol) in 1987, marketed under brand names such as Actigall®, UDCA's historical origin traces back to its first isolation from the bile of the Asian black bear (*Ursus thibetanus*), from which the 'urso-' prefix is derived. This legacy is still reflected in registered trademarks used for commercial formulations such as Ursofalk®, Ursogal®, Cholurso®, Ursogrix® or Urso 250®¹.

Over the last 30 years, Dipharma has developed, scaled up, and industrialized multiple bile acid derivatives APIs and related compounds. This sustained commitment has led to the development of IP-protected routes, impurity-controlled processes, and GMP-scale manufacturing capabilities that span the entire lifecycle of the cholane framework.

The following timeline captures Dipharma's continuous innovation in this domain.

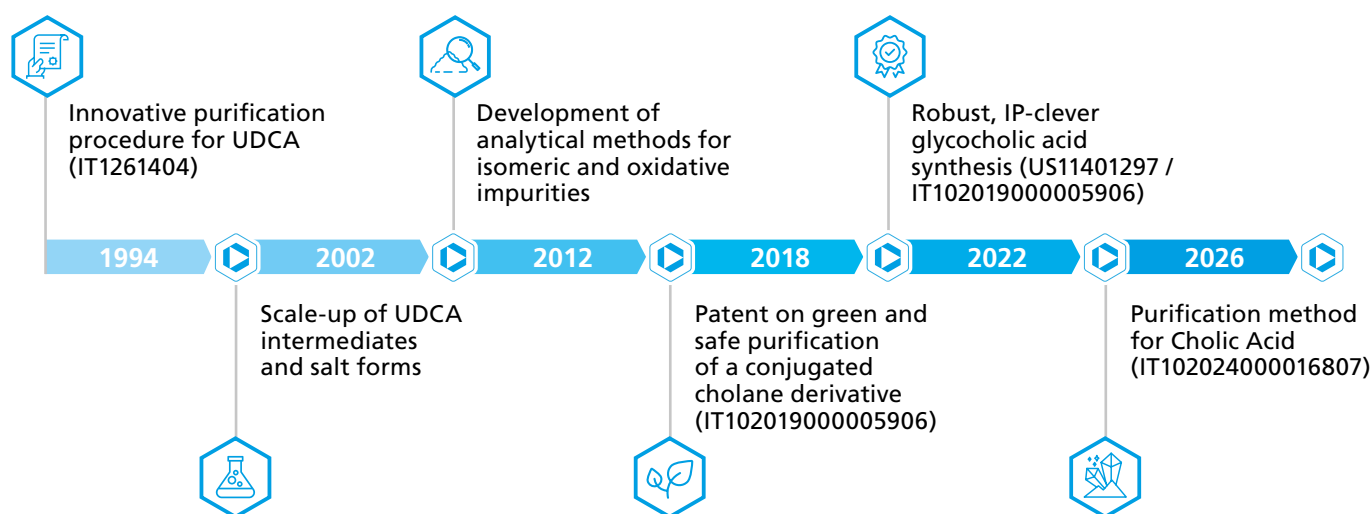


FIGURE 1: Dipharma and the Cholane Scaffold: A 30-Year Timeline.

In this white paper, we provide an integrated view of Dipharma's expertise in cholane chemistry, combining historical know-how, modern synthesis approaches, regulatory insight, and real-world impurity management. This knowledge is key not only to delivering compliant generics but also to supporting next-generation bile acid-based therapeutics.

Market Overview of FDA-Approved Cholane-Structured Bile Acid Derivatives

The global market for FDA-approved cholane-structured bile acids (UDCA, CDCA, cholic acid, deoxycholic acid, and obeticholic acid) exhibits stable, long-term demand driven by chronic and orphan indications, with **ursodeoxycholic acid (UDCA) dominating > 80% of global volumes and revenues**, reflecting its established role as the standard of care in cholestatic liver diseases. **Chenodeoxycholic acid (CDCA)** and **cholic acid** are characterized by significantly lower volumes, consistent with their use in rare inherited bile acid synthesis disorders. In recent years, CDCA has regained clinical relevance through regulatory updates and expanded access for cerebrotendinous xanthomatosis (CTX), while cholic acid (Cholbam®) remains positioned within the orphan drug framework for pediatric and metabolic indications.

Although **obeticholic acid (OCA)** represents a marginal share in terms of volume, its high unit price reflects a niche, high-value positioning, historically linked to second-line treatment of primary biliary cholangitis. Importantly, while OCA received accelerated FDA approval, it has not obtained full approval for MASH/NASH indications, and its marketing authorization was withdrawn in the EU in 2024, underscoring the regulatory complexity associated with next-generation bile acid derivatives. From a regional perspective, Asia (including China and Southeast Asia) accounts for a substantial share of global UDCA volumes, while Europe and North America represent mature, stable markets with stringent quality and regulatory expectations. CDCA and cholic acid markets remain geographically concentrated, reflecting the localization of rare disease treatment centers and reimbursement frameworks. Looking ahead, the market for bile acid derivatives is expected to maintain steady growth, driven by stable demand in rare disease indications

and increasing recognition of their broader metabolic effects. Importantly, **the pipeline of bile acid-based molecules is gaining momentum**, with multiple derivatives currently in **clinical development for high-prevalence indications such as metabolic dysfunction-associated steatohepatitis (MASH), type 2 diabetes, and obesity**. The identification of bile acids as ligands for receptors like FXR (Farnesoid X Receptor) and **TGR5** (G protein-coupled bile acid receptor 1) has opened new therapeutic pathways targeting inflammation, lipid metabolism, and insulin sensitivity². This evolving scientific landscape highlights a strong potential for **expansion beyond the traditional orphan niche**, suggesting that the cholane-derivatives market could undergo a substantial transformation in the coming years from a specialized sector into a broader therapeutic frontier.



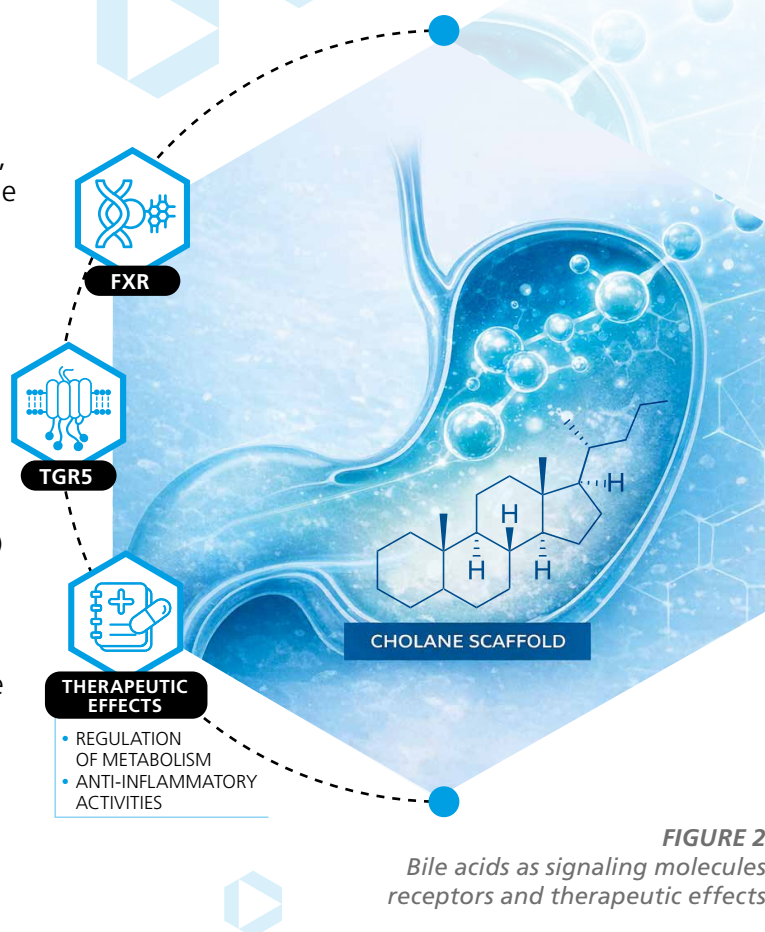
Cholane Framework Chemistry: A Pharmacological Renaissance

Bile acids were once seen as mere detergents, amphipathic molecules evolved to aid fat digestion in the small intestine. Today, they are recognized as pleiotropic **signaling molecules**, capable of modulating gene expression, immune responses, glucose metabolism, and even cell proliferation.

This shift in perception has been driven by the discovery of their role as **endogenous ligands** for a set of nuclear and membrane receptors, most notably:

- **FXR** (farnesoid X receptor)
- **TGR5** (G protein-coupled bile acid receptor 1)
- **VDR** (vitamin D receptor)
- and more recently, **S1PR2** (sphingosine-1-phosphate receptor 2), **PXR** (pregnane X receptor), and **GPBAR1** (G protein-coupled bile acid receptor 1)

These interactions have sparked a new era in drug design, with bile acid scaffolds being modified, conjugated, and hybridized to generate novel therapeutics.



Pharmacologically Active Bile Acid Derivatives

Structurally, bile acids share a **steroid nucleus (cyclopentanoperhydrophenanthrene)** with various substitutions at positions C-3, C-7, C-12, and the side chain. Their diversity in function stems from minor variations:

- Hydroxy epimers (7 α vs. 7 β)
- Oxidized ketones at C-7
- Conjugation with taurine or glycine
- Side-chain derivatization (e.g., 6-ethyl in OCA)

This subtle chemistry translates into **distinct and highly differentiated pharmacological profiles**, as seen in the growing list of bile acid-related drug approvals.

Once dismissed as simple detergents aiding lipid absorption, bile acids have undergone a

pharmacological renaissance. They are now recognized as endogenous ligands for a wide array of nuclear and membrane receptors—most notably **FXR**, **TGR5**, and **VDR**—with systemic effects on metabolism, immunity, and inflammation. This shift in perception has led to a surge in bile acid-based therapeutics, including:

- **UDCA (ursodeoxycholic acid)**, the gold standard for primary biliary cholangitis (PBC).
- **OCA (obeticholic acid)**, a semi-synthetic FXR agonist used in PBC and under investigation for NASH.
- **TUDCA (tauroursodeoxycholic acid)**, an emerging neuroprotective and mitochondrial stabilizer.
- **CDCA (chenodeoxycholic acid) and cholic acid**, essential for rare pediatric indications.

A Approved API

Approved drug (Brand)	Status & Year (US only)	Indication	Scaffold Type
Chenodeoxycholic acid (Chenix)	FDA-approved [1983]	Treatment of gallstones	CDCA
Ursodeoxycholic acid (UDCA, Actigall / URSO)	FDA-approved [1987]	Primary biliary cholangitis (PBC)	7β
Deoxycholic acid (Kybella)	FDA-approved 2015	Injectable treatment that destroys fat cells in the treatment area under the chin to improve your profile	Deoxycholic acid
Cholic acid (Cholbam / Orphacol)	FDA-approved 2015 (cholic acid)	Bile acid synthesis disorders	Cholic acid

B Investigational/Failed

Investigational / Failed	Status & Year (US only)	Indication	Scaffold Type
OCA in NASH (COBALT trial)	Withdrawn from US market in 2025 for PBC; Not approved for NASH/MASH	NASH and PBC (2nd line)	6α-ethyl CDCA
TUDCA	Investigational*; previously part of RELYVRIO (withdrawn 2024) ALS (combination), investigated also for MASH, T2D, NDDs	ALS	UDCA + Taurine
Aramchol (FABAC)	Phase II; not yet approved	NASH/liver fat reduction	Cholic acid + fatty acid

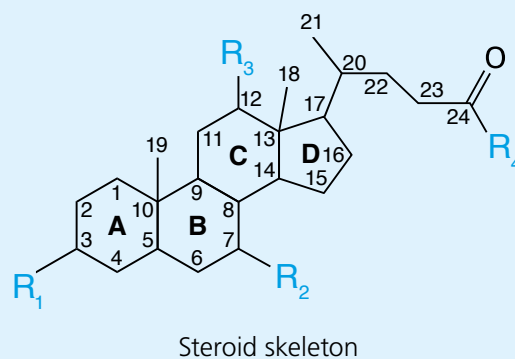
* Please note that while TUDCA shares the cholane framework, it has not received centralized approval from FDA or EMA as a standalone hepatobiliary treatment. However, in certain EU Member States (e.g., Italy), tauroursodeoxycholic acid is authorized under national marketing authorizations for hepatobiliary indications. TUDCA gained broader international attention when used in combination (RELYVRIO) for ALS, before its withdrawal in 2024, and continues to be investigated in metabolic and neurodegenerative conditions.

C Excipient

Excipient	Activity	Indication	Scaffold Type
Cholylsarcosine	Absorption enhancer	Peptide drug delivery (investigational)	Cholic acid derivative
Glycocholate sodium, Glycocholic acid	Absorption enhancer	Formulation excipients	Cholic acid conjugates

TABLE 1: Key cholane-structured bile acid derivatives: a snapshot of approved drugs and emerging compounds shaping the therapeutic landscape.

The pharmacological plasticity of the cholane scaffold explains the increasing demand for well-characterized, GMP-grade bile acid derivatives. However, manufacturing these compounds is not trivial. The rigidity of the steroidal backbone, the sensitivity of functional groups, and the presence of isomeric and oxidative impurities all pose unique challenges.



Name	R ₁	R ₂	R ₃	R ₄
Cholanic acid	H	H	H	OH
Cholic acid	OH	OH	OH	OH
Chenodeoxycholic acid	OH (α)	OH (α)	H	OH
Deoxycholic acid	OH	H	OH	OH
Ursodeoxycholic acid	OH (α)	OH (β)	H	OH
Lithocholic acid	OH	H	H	OH
Glicocholate	OH	OH	OH	NHCH ₂ COO ⁻
Taurocholate	OH	OH	OH	NHCH ₂ CH ₂ SO ₃ ⁻

TABLE 2: Structures of the most abundant bile acids in humans, and their glycine and taurine conjugates.

Dipharma's Evolution in Cholane Chemistry: A Strategic Continuum

Dipharma's journey into bile acid chemistry began in the early 1990s, at a time when cholane derivative APIs were mostly limited to natural conjugates used in hepatobiliary therapies. Over three decades, the company has built an industrial and scientific platform that has evolved in parallel with:

- The diversification of bile acid derivatives used as APIs or excipients.
- The tightening of impurity specifications and genotoxicity risk assessments.
- The increased complexity of regulatory submissions.

What began as a focused effort to produce conjugated and differently functionalized bile acid derivatives has evolved into an integrated expertise encompassing API development, analytical control, regulatory justification, and GMP-compliant scale-up. This continuity is not accidental: it reflects a deliberate corporate strategy to invest in **difficult molecules that require long-cycle know-how**, and to **cultivate cross-functional capabilities** that create value far beyond synthetic chemistry alone.

Three Decades of Cholane Chemistry Know-How

The long-term commitment to cholane scaffolds has enabled Dipharma to:

- Develop proprietary methods for handling **amphiphilic, low-solubility compounds**.
- Master the **solid-state behavior** of bile acid derivatives (e.g., polymorphs, solvates, filtration).
- Build an internal **impurity database** that supports faster method development and lifecycle management.
- Train regulatory and quality teams to deal with **API-specific issues** such as isomeric profiling, bile source variability, and impurity qualification.

Area	Practical Experience/Milestones
Analytical Development	Design of HPLC methods enabling early detection and separation of positional isomers of UDCA and CDCA
Impurity Profiling	Internal impurity libraries developed to identify and control typical cholane-related degradants and side-products
Patent Strategy	Development of better and new synthesis routes for UDCA and cholane conjugates, and their protection, with the goal of obtaining freedom to operate and preventing third parties from using Dipharma technology
Regulatory Justification	Experience in qualification of bile acid impurities (e.g. DCA, LCA, isomers) aligned with Ph. Eur., USP and EMA expectations
Process Innovation	Implementation of greener oxidation steps and control of isomeric conversion during process scale-up

TABLE 3: Key Capabilities Acquired through Cholane API Projects.

Analytical & Regulatory Expertise: Controlling Complexity

In the field of bile acid derivative APIs such as ursodeoxycholic acid (UDCA), mastering impurity control is not just a regulatory requirement, it's a technical imperative. The complexity of these molecules, with multiple chiral centers and oxidative functionalities, creates a fertile ground for **isomeric impurities, oxidative by-products, and side-chain rearrangements**.

Dipharma has invested heavily in developing **robust, highly selective analytical methods** that go beyond compendial requirements, ensuring the comprehensive detection and control of all relevant impurities, even at trace levels. These methods support both **regulatory compliance** and **process understanding**, bridging development with lifecycle control.

Structurally Relevant Cholane-related Impurities

The table below summarizes the most critical structurally related bile acid impurities encountered during UDCA synthesis and stability testing. These impurities are either:

- **Process-related** (arising from incomplete conversions or reagent selectivity).
- **Raw material-related** (from bile-derived starting materials).
- **Degradants** (formed under stress or long-term storage conditions).

Compound	Role	Structural Origin	Analytical Relevance
Chenodeoxycholic acid (CDCA)	Intermediate/ Impurity A	7 α -epimer of UDCA	Often present in bile-derived raw materials
Cholic acid (CA)	Starting material/ Impurity B	Trisubstituted bile acid	Its persistence indicates incomplete purification
Lithocholic acid (LCA)	Degradation product/ Impurity C	Monohydroxy acid	Associated with toxicological concern
Deoxycholic acid (DCA)	Side-product/ Impurity E	Dihydroxy acid	Regulated to $\leq 0.8\%$ in Ph. Eur.
Isoursodeoxycholic acid	Isomer/Impurity H	C-3 epimer of UDCA	Critical isomeric impurity; requires baseline resolution
IsoCDCA	Minor isomer	From non-selective reduction	May co-elute with other bile acids
3α,7-ketodeoxycholic acid	Intermediate/ by-product	Incomplete reduction of CDCA	Requires specific detection (see case study)
UDCA methyl ester	Artifact	Formed during HPLC under acidic MeOH	Demonstrates the need for pH control in analytics

TABLE 4: Structurally Related Impurities in UDCA Manufacturing and Control.

Regulatory Alignment and Method Development

Dipharma's analytical approach is fully aligned with:

- **ICH Q3A/B** guidelines for impurity profiling.
- **European Pharmacopoeia (Ph. Eur.)** monograph on UDCA.
- **FDA expectations** for bile acid-based APIs, where impurity qualification below the identification threshold is often required for structurally similar molecules.

To meet these requirements, Dipharma has developed and validated:

- **Specific HPLC methods** with optimized selectivity and run times.
- **Custom reference standards**, synthesized or purchased where necessary.
- **Stress studies** to predict impurity formation under worst-case scenarios.
- **Impurity tracking matrices** to support DMF and CEP submissions.

Case in Point Anticipating the 2026 UDCA Monograph Revision

While ursodeoxycholic acid (UDCA) has been marketed for decades, regulatory expectations continue to evolve. A revised version of the European Pharmacopoeia monograph for UDCA was published in January 2026, introducing significant analytical updates, such as a dedicated HPLC method for impurity J (7 β -hydroxy-5 β -cholan-24-oic acid) and tighter controls on structurally related bile acid impurities. Dipharma anticipated these

changes early and proactively and initiated internal method development and impurity qualification before the revised text was officially adopted. This forward-thinking approach ensures that our analytical and manufacturing frameworks remain aligned with evolving regulatory standards, giving our partners long-term confidence and compliance. (Source: *Pharmeuropa* 36.2 and *Ph. Eur.* 12.1, Monograph 1275)

Case Study → Identification of Isocholic Acid (RRT 0.68) in UDCA via QC Investigation

During routine HPLC analysis of UDCA using the European Pharmacopoeia (Ph. Eur.) method for related substances, a minor but well-defined peak was consistently observed at relative retention time (RRT) 0.68, not assigned to any listed pharmacopoeia

impurity. To ensure its correct identification, an in-depth analytical study was conducted using both the Ph. Eur. and United States Pharmacopoeia (USP) HPLC methods.

Experimental Setup and Findings

A targeted spiking study was performed using a certified isocholic acid (ICA) reference standard.

Under the USP method, the RRT 0.68 peak in the UDCA sample matched both the retention time and UV spectrum of the isocholic acid standard. Quantification by external standard calibration gave typical levels of ~0.07% in commercial batches, below the Ph. Eur. threshold for unspecified impurities.



Analytical Investigation

To confirm the identity of the RRT 0.68 impurity, the QC laboratory prepared and analyzed four solutions under the USP HPLC method for UDCA related substances:

- **Blank solution**, solvent mixture only, to verify baseline purity.
- **Standard solution (0.10% isocholic acid)**, prepared from a certified reference standard to establish retention time and peak area correlation.
- **UDCA sample (unspiked)**, representative commercial batch, analyzed to quantify the naturally occurring impurity.
- **UDCA + standard spike**, same UDCA sample with addition of the isocholic acid standard, to confirm peak identity by co-elution.
- The RRT 0.68 peak in the unspiked sample perfectly matched both the retention time and UV spectrum of the isocholic acid standard. In the spiked sample, peak areas were additive, confirming the assignment.

Sample Description	Calculated Content (%)*	Notes
UDCA sample (unspiked)	0.070	Naturally occurring impurity
Standard solution (0.1%)	0.100	Reference for quantification
UDCA + standard spike	0.147	Confirmed additivity

TABLE 5: Impurity Spiking and Recovery Summary.

* Data derived from internal QC investigation using the European Pharmacopoeia HPLC method for UDCA related substances.

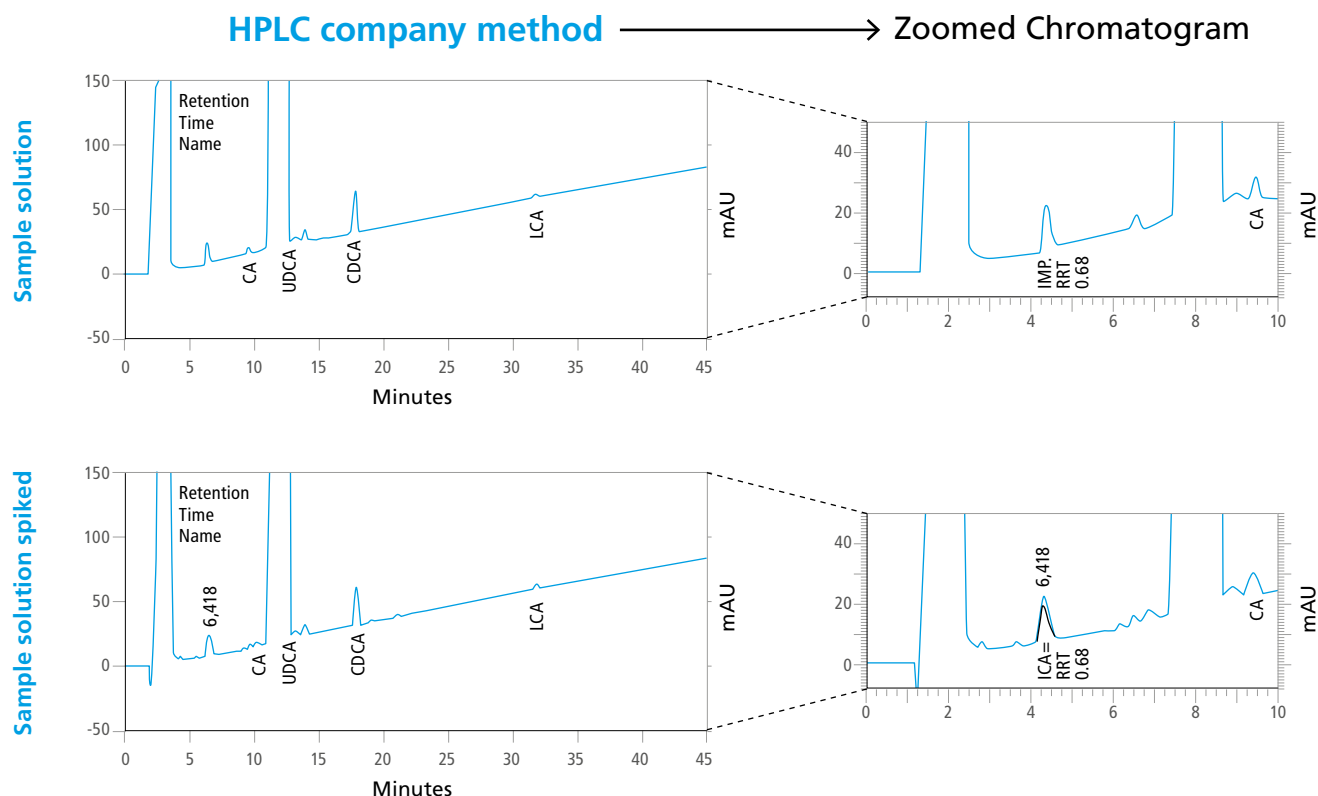


FIGURE 3: HPLC overlay of spiked vs. unspiked UDCA sample, highlighting the peak at RRT 0.68.

This impurity level is below the Ph. Eur. threshold ($\leq 0.10\%$) but is **structurally close to UDCA**, underscoring the importance of extended peak tracking, especially in **bile acid-rich matrices**.

Impurity Origin and Relevance

Isocholic acid differs from structurally related bile acids such as 3 α ,7-ketodeoxycholic acid only by the oxidation state at C-7, making it difficult to distinguish when chromatographic resolution is limited. The improved separation achieved with the USP method ($\Delta RT \sim 0.6$ min vs. ~ 0.1 min in the Ph. Eur. method) was critical for correct identification.

In the UDCA synthesis process, traces of cholic acid may carry over its 3-keto derivative, which upon reduction generates isocholic acid.

Takeaway

The confirmed identification of isocholic acid at RRT 0.68 demonstrates the value of orthogonal analytical approaches in bile acid chemistry, delivering both regulatory confidence and a deeper understanding of impurity origins within complex cholane frameworks.

Dipharma's Contributors

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- ▷ **Sabrina Furin** *Director of Corporate QA & RA*
- ▷ **Paola Strola** *QA Manager*

Integrating Analytical Control into Process Development

The data gathered from impurity profiling is not just used to release product; it feeds directly back into process improvement:

- **Reduction steps** were fine-tuned to minimize incomplete conversion.
- **Raw material controls** were tightened to monitor cholic acid content.
- **Crystallization procedures** were adjusted to purge isomeric species.

This integration of **analytical feedback** into chemical development is a hallmark of Dipharma's approach to high-complexity APIs.

From Lab Bench to GMP: Dipharma's Cholane Chemistry in Action

Beyond strategic continuity, Dipharma's strength in bile acid derivative APIs lies in its **technical execution**. Developing scalable, compliant, and robust processes for molecules such as UDCA, CDCA, and their derivatives requires more than familiarity with classical organic reactions. It requires:

- A detailed understanding of **stereoelectronic effects** on reactivity and selectivity.
- The ability to manage **highly viscous, low-solubility intermediates** at scale.
- The foresight to anticipate **regulatory or safety pitfalls** before they occur.

Mastering Redox and Isomerization Chemistry

Many bile acid syntheses involve delicate **oxidation–reduction cascades** aimed at selectively transforming hydroxyl groups or controlling stereochemistry at C-7. This includes:

- Selective oxidation of CDCA to 7-keto intermediates.
- Epimerization techniques to convert CDCA into UDCA.
- Avoiding over-reduction or formation of isoursodeoxycholic acid (isoursodeoxycholic acid) and isoCDCA impurities.

Dipharma has implemented a toolbox of **mild oxidants** (e.g., TEMPO, Dess–Martin) and **stereoselective reduction conditions** (e.g., $\text{NaBH}_4/\text{CeCl}_3$, catalytic hydrogenation) to optimize yield and control.

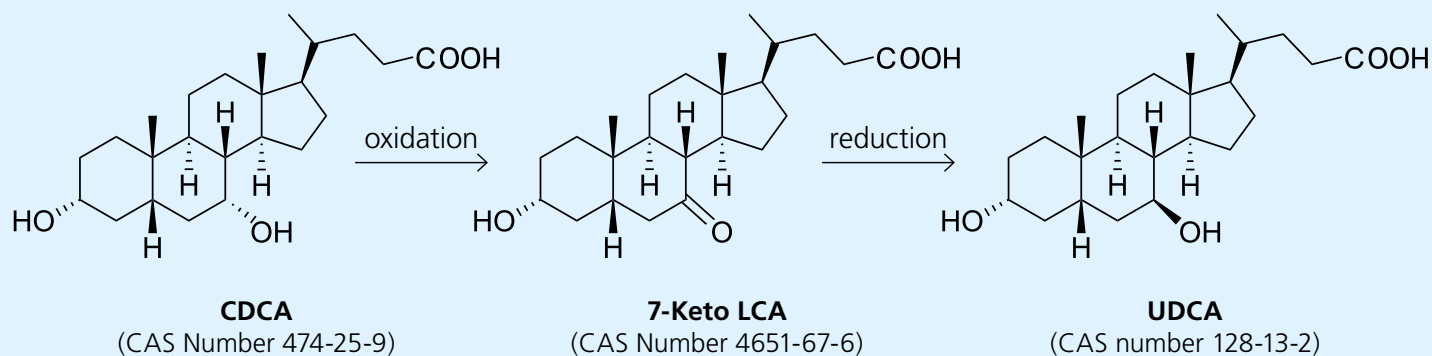


FIGURE 4: General scheme for core step of UDCA synthesis.

Green and Safer Process Design

To address both environmental and regulatory expectations, Dipharma has systematically replaced hazardous reagents and optimized process safety. Examples include:

- **Safer, solvent-driven purification strategies for conjugated bile acids.**

For taurine-conjugated bile acids such as TUDCA, Dipharma developed purification routes based on controlled extraction and precipitation using common solvent systems (e.g., alcohols and ketones) and protic acids, achieving high purity while minimizing harsh chemical treatments and reducing operator exposure to reactive reagents. This approach supports robust impurity control and scalable isolation under GMP conditions. (IT102019000005906)

- **Crystallization and solid-state selectivity as a green lever.**

Where feasible, Dipharma leverages crystallization and solid-liquid selectivity to separate closely related bile acids (e.g., UDCA vs CDCA), limiting the need for reagent-intensive transformations and enabling efficient downstream processing. Such 'physical' separation approaches can reduce waste streams and simplify purification in industrial settings. (IT102000900849186)

Calorimetry and risk assessments have been integrated into route selection and scale-up, ensuring **safe processing at pilot and plant levels.**

Integrating Chemistry with Impurity Control

The success of a cholane derivative synthesis does not stop at yield. Due to the **close structural similarity among bile acid isomers**, Dipharma integrates:

- In-process controls to monitor key isomeric species.
- Real-time feedback from QC and Analytical R&D.
- Systematic fate and purge studies for critical impurities (e.g., lagocolic acid, isoCDCA).

Process robustness is confirmed through repeated pilot campaigns and full-scale GMP validation batches, with **impurity profiles mapped in detail across the process train.**

Patent-Driven Innovation

Unlike many API suppliers who rely on public-domain methods, Dipharma has developed a suite of **proprietary, patent-protected technologies** for bile acid synthesis. Some examples are included in the table below:

Decade	Focus Area	Key Patents / Milestones	Remarks
1980s - 1990s	Classical amidation	IT1261404, US5565587, US5508453	Taurine/glycine conjugates
1990s - 2000s	Synthesis of UDCA from CDCA	EP0424232	Epimerization & reduction platforms
2010s	Identification of new sources for bile acids	US7982062	An increased demand for bile acid derivatives as drugs necessitates to identify suitable new sources in view of limited availability of bovine bile
2020s	Purification	US11401297	Synthesis and purification of glyco-derivatives of cholic acid

TABLE 6: Chronological View of Cholane Innovation at Dipharma.

Dipharma's experience is not limited to a single API or route. It spans across:

- **API-grade bile acids (UDCA, CDCA)**
- **Key intermediates (7-keto-CDCA, isoUDCA)**
- And **novel investigational scaffolds**, with flexibility to adapt processes under CDMO setups.

This technological continuum makes Dipharma a reference partner for any company aiming to commercialize cholane scaffolds with confidence, quality, and innovation at the core.

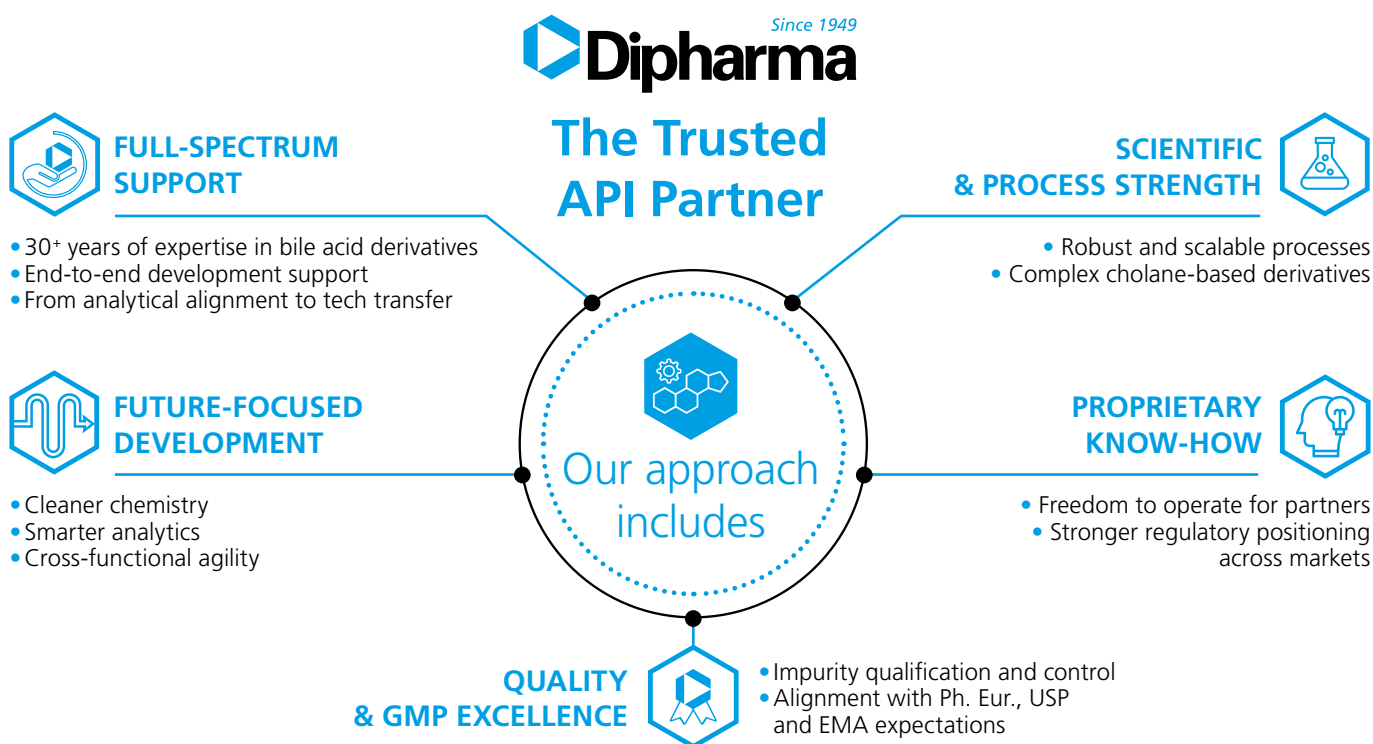
Conclusions & Outlook

The cholane framework represents one of the most deceptively complex frameworks in medicinal chemistry. With its rigid polycyclic backbone, reactive hydroxyl topography, and susceptibility to isomerization, it poses challenges from synthesis to scale-up, and from impurity profiling to regulatory defense.

At Dipharma, we have not only embraced the complexity of cholane chemistry, we have made it our domain.

Over more than three decades, our teams have designed and validated **robust, scalable processes** for both established and niche bile acid derivatives. We've built a proprietary body of know-how that grants our partners **freedom to operate** and delivers **regulatory resilience** across geographies. Whether developing impurity standards, navigating pharmacopoeial updates, or managing tech transfers, **Dipharma brings full-spectrum support from route design to GMP release**.

And we continue to invest in **cleaner chemistry, smarter analytics**, and **cross-functional agility** that anticipates the needs of tomorrow's cholane-structured compounds with new therapeutic applications.



References:

- [1] Shoda et al. Hepatology, 1999; 30, 6.
- [2] Chiang J.Y.L et al., Physiological Reviews, 2013; 93(4): 1479–1517.

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www.dipharma.com
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