SUMMARY
ABSTRACT

A simple protocol involving the use of a Soxhlet extraction followed by saponification, solvent crystallization and column chromatography was developed for isolation of solanesol from tobacco (*Nicotiana tabacum* L.). The extraction procedure was compared with cold extraction in terms of efficiency and recovery. A simple RP-HPLC method for determination of solanesol was developed, validated and adopted. The isolated solanesol was subjected to spectral analysis viz., IR, $^1$H NMR, $^{13}$C NMR and Mass. Coenzyme Q$_{10}$ was synthesized, starting from solanesol, with Coenzyme Q$_0$ and decaprenol or isodecaprenol as the key intermediates. The chromatographic conditions were optimized for separation of process - related impurities from CoQ$_{10}$ by NARP-HPLC. The impurities were characterized using modern spectroscopic techniques viz., $^1$H NMR and APCI-MS. HPLC-PDA and HPLC-ELSD were also employed for the detection of CoQ$_{10}$ and its process impurities. The method for analysis of CoQ$_{10}$ in bulk drugs and pharmaceuticals was validated in terms of accuracy, precision, linearity, LOD and LOQ.

Isolation and Characterization of Solanesol from *Nicotiana tabacum* L.

The current state of the art on isolation, purification and determination of solanesol from tobacco are presented. In the present work, an economical and efficient protocol for isolation as well as purification of solanesol from tobacco using Soxhlet extraction, followed by column chromatography, saponification and/or recrystallization was described. The soxhlet extraction is more suitable for isolation of solanesol on a laboratory scale and high purity of 95–98% solanesol was produced using common laboratory chemicals. The procedures followed for purification of solanesol from the crude extracts of tobacco are presented in Fig.5.1.
Tobacco/Tobacco Waste

Soxhlet Extraction/Large Scale Extraction

Hexane 4hr

Crude Solanesol (20%)

Silica Gel Column (93% Solanesol)

Hexane Recrystallization (96% Solanesol)

Saponification

Hexane Extract (45% Solanesol)

Acetone (Insoluble matter removed) & Recrystallization

Silica Gel Column (95% Solanesol)

Methanol Recrystallization

Hexane Recrystallization (96-98% Solanesol)

Acetonitrile Recrystallization (2 times) (90% Solanesol)

Fig. 5.1. Flow sheet of procedures followed for purification of solanesol from the crude extract of tobacco.
The analysis of published data revealed that the HPLC is an effective tool for determination of solanesol in tobacco compared to gas chromatography due to the time consuming derivatization steps involved in GC. However, most of the earlier methods reported the use of normal phase LC probable due to the hydrophobic nature of solanesol. Further the development and validation of a non-aqueous reverse phase HPLC method on a C_{18} column using isopropyl alcohol : methanol (60:40) as a mobile phase and UV detection at 210 nm for determination of solanesol in tobacco was described.

The HPLC method was validated with respect to precision (inter and intra-day assay of solanesol, RSD < 1%) and accuracy (± 5%). The assay precision was < 5%, and accuracy was > 98%. Linearity (range 0.1-1.25 mg/ml), mean equation of the calibration curve (n=6) obtained from six points way \( y = 13,473,671x + 1,560,765 \) and a regression coefficient (0.9996) were established. The measured LOD and LOQ values were 0.2µg/ml and 0.7µg/ml, respectively.

The developed RP-HPLC method was used for determination of solanesol in different tobacco samples (0.43-1.01%). The method was adopted in the analysis of crude/enriched extracts and purified samples during extraction of solanesol. The extracted solanesol was characterised by spectral analysis viz., IR, \(^{1}H\) NMR, \(^{13}C\) NMR and Mass.

**Synthesis of Ubiquinone Q_{10} (CoQ_{10}) from Solanesol**

Coenzyme Q_{10} (CoQ_{10}), a member of the ubiquinone family, is an essential component of the mitochondrial electron transfer chain. It is widely being used for cardiac health and also as an antioxidant. It also holds promise as an anticancer agent. The commercial quantities, however, limited as the CoQ_{10} in the market is solely derived from the fermentation technology and the available synthetic methodologies are not commercially feasible. The present study has been an attempt towards the cost viable synthesis of ubiquinone Q_{10}. A new
synthetic route for the preparation of ubiquinone $Q_{10}$ ($CoQ_{10}$) is described. Ubiquinone $Q_{10}$ ($CoQ_{10}$) is a potentially useful compound having wide number of health applications especially those related to cardiovascular diseases. The present process achieves $CoQ_{10}$ starting from relatively inexpensive precursor, called isoprenol. It achieves key synthetic intermediate decaprenol, needed for the expansion of the prenyl chain, through a novel and viable process. This is more economical than expanding expensive natural nonaprenyl compound called solanesol by an isoprene unit before coupling to the $Q_0$ precursor.

We have carried out Coenzyme $Q_{10}$ ($CoQ_{10}$) synthesis in which decaprenyl bromide (9) was the intermediate, obtained from two routes as shown in the following Fig.5.2.

Solanesol (1) on bromination afforded the compound solanesyl bromide (2) which on treatment with ethyl acetoacetate followed by hydrolysis and decarboxylation gave the ketone (3). The keto compound (3) on reacting with sodium acetylide in ammonia at $-78^0C$ afforded acetylenic alcohol (4). This alcohol, on partial reduction with Lindlar’s catalyst provided isodecaprenol (5) within 92% yield. In order to obtain decaprenol (8), solanesyl bromide (2) was treated with sodium-p-toluene sulphinate in DMF to give solanesyl sulphone (6). On coupling solanesyl sulphone (6) with the catalyst, bromo compound (12) (4-bromo-3-methyl-2-butenyl acetate) afforded C$_{50}$ coupled product (7), which, on reductive cleavage with 4% Na(Hg) gave C$_{50}$ alcohol (8). Either isodecaprenol (5) or decaprenol (8) on bromination gave decaprenyl bromide (9), coupling with hydroquinone ($CoQ_0$) and oxidation afforded Coenzyme $Q_{10}$ (10) with 57.5% yield as an orange powder.

The procedure followed for the synthesis of Coenzyme $Q_{10}$ ($CoQ_{10}$) from solanesol was presented in Fig.5.2.
We can conclude that our strategy gave all *trans*-polyprenyl alcohols which were confirmed by NMR. The coupling of all *trans*-polyprenyl bromides and 2,3-dimethoxy-5-methyl-1,4-hydroquinone (CoQ₀) was achieved by calcium oxide, in chloroform, followed by oxidation with aqueous ferric chloride afforded all *trans*-Coenzyme Q₁₀ (CoQ₁₀). The PMR, IR and Mass were in accordance with the spectral data. Thus a better cost effective strategy was developed.

**Figure 5.2. Flow sheet of procedure followed for the synthesis of Coenzyme Q₁₀ (CoQ₁₀) from solanesol.**
Summary

The spectral analyses of coenzyme Q$_{10}$ (CoQ$_{10}$) and intermediate compounds were carried out by IR, UV, $^1$H NMR, $^{13}$C NMR & Mass values for characterization.

Characterization and Determination of Ubiquinone (Coenzyme Q$_{10}$) and the process related impurities

An isocratic HPLC method for monitoring of process related substances of Coenzyme Q$_{10}$ (V) and its related substances (I) 2,3-dimethoxy-5-methyl-p-benzoquinone (I), solanesol (II), solanesyl acetone (III), isodecaprenol (IV) as shown in Fig.5.3. A C$_8$ column with acetonitrile and isopropyl alcohol (84:16, v/v) as a mobile phase at a flow rate of 1.0ml/min and detection at 210 nm using photo diode array (PDA) was used. The effect of organic modifier (isopropyl alcohol 15 to 30%), temperature of column (15–50 $^0$C) on retention and resolutions were studied to optimize chromatographic conditions.
Fig. 5.3. Chemical structures of CoQ₁₀ (V) and its related substances (I) 2,3-dimethoxy-5-methyl-p-benzoquinone, (II) Solanesol, (III) Solanesyl acetone & (IV) Isodecaprenol.
Different batches of Coenzyme (CoQ\textsubscript{10}) were analyzed by HPLC and the method was validated with respect to precision (inter and intra-day assay of CoQ\textsubscript{10}, RSD < 1%), accuracy (99.2 – 100 with RSD 0.05-0.47% for CoQ\textsubscript{10} and 95.10 – 101.02 with RSD 0.29 – 1.35% for impurities). Linearity (range 50-300\textmu g/ml with $r^2 \geq 0.9999$ for CoQ\textsubscript{10} and 0.25 - 2\textmu g/ml with 0.9981 for impurities), limit of detection (LOD), limit of quantification (LOQ) and specificity. The developed method was found to be selective, sensitive, accurate and precise. The method was applied to determine CoQ\textsubscript{10} and process-related substances in bulk drugs and pharmaceutical formulations. The developed method was validated as per ICH guidelines. The spectral analyses of Coenzyme Q\textsubscript{10} (CoQ\textsubscript{10}) and related compounds were identified by $^1$H NMR & APCI-Mass.