Section 10.0

CONCLUSION ON ALL CHAPTERS

The analytical development and quality control laboratories in pharmaceutical industries require analytical methods that predict accurate and precise data of the drug content. The aim of the thesis work was to develop and validate simple, rapid, accurate, precise, robust and rugged analytical methods for the determination of eight anti-diabetic drugs, by adopting simple and cost-effective techniques like titrimetry, UV/visible spectrophotometry and advanced techniques such as HPLC and UPLC.

Because of the unique features of chemical methods like titrimetry and visible spectrophotometry, the author has employed these techniques for the assay of one and three organic compounds, respectively, having anti-diabetic therapeutic activity. Not to be left behind in the use of modern physical methods, the author has also employed UV-spectrophotometry, HPLC and UPLC, the most widely used assay techniques in pharmaceutical industries today, in this study. This has resulted in the culmination of development and validation of old (chemical) and newer (physical) methods for the eight drugs namely, repaglinide (RPG), metformin hydrochloride (MFH), pioglitazone hydrochloride (PGH), nateglinide (NTG), glipizide (GPZ), rosiglitazone (ROS), miglitol (MGL) and chlorpropamide (CLP). The proposed UPLC and HPLC methods are highly sensitive, stability-indicating, have wide linear dynamic ranges and shorter analysis run time. The developed methods were applied to the determination of active drug in bulk form, tablet preparations and a few methods in spiked human urine. No interferences were observed by inactive ingredients present tablets and biochemical substances present in urine. These further confirm the excellent selectivity of the proposed methods.

Two UV- and four visible spectrophotometric, one each of titrimetric and UPLC methods were developed for the assay of repaglinide (RPG) in bulk drug, its pharmaceuticals, and validated according to ICH guidelines. Two UV-spectrophotometric methods were developed using 0.1M NaOH and 0.1M HCl as diluents and the vulnerability of the drug to various stress conditions was studied. The outstanding performance characteristics of the proposed methods are
simplicity, high sensitivity and use of inexpensive chemicals. The electron donating ability of tertiary aliphatic amine group of RPG was exploited to develop visible spectrophotometric methods based on ion-pair and charge-transfer (CT) complex formation reactions. Ion-pair reactions utilize two sulphophthalene dyes; bromocresol purple and bromocresol green. \( p \)-Chloranilic acid and 2,3-dichloro-5,6-dicyanoquinone are the two CT reagents used to develop two simple and moderately sensitive methods. The developed spectrophotometric methods are rapid, extraction-free, and do not involve heating/cooling steps and all the measured colored species are stable for up to 5 hours which is more than enough to acquire reproducible readings and superior to all existing spectrophotometric methods. The proposed UPLC method is sensitive, has wide linear dynamic range and shorter analysis run time. This is the first stability-indicating UPLC method for RPG. The reported HPLC methods are time consuming and less sensitive compared to the proposed UPLC method. The solvent consumption is 2-3 times less than that of the reported HPLC methods. The titrimetric method is based on the neutralization reaction of the aliphatic tertiary amino group of RPG with acetic perchloric acid as titrant in anhydrous acetic acid medium using crystal violet as indicator. The reaction stoichiometry of 1:1 (drug:titrant) was obtained which served as the basis for calculation. The assessment of the developed methods was made at the end of the chapter.

For metformin hydrochloride (MFH), six spectrophotometric and one UPLC methods were developed and validated. Four spectrophotometric methods are based on the measurement of formed CT complexes with 2,4-dinitro phenol, picric acid, \( p \)-chloranilic acid (CAA) and 2,3-dichloro-5,6-dicyanoquinone (DDQ). Two methods using CAA and DDQ were successfully applied to spiked human urine with satisfactory recovery. The proposed two ion-pair spectrophotometric methods employed bromophenol blue and bromothymol blue as ion-pair reagents. All proposed visible spectrophotometric methods are extraction-free, simple and sensitive when compared to most visible spectrophotometric methods reported so far. All the colored measured species are stable for more than 3 hours which is more than enough to acquire reproducible readings. The UPLC method was found to be sensitive, specific for MFH with
wide linear dynamic range and shorter analysis time. This is the first stability-indicating UPLC method developed for MFH.

One UV-spectrophotometric, two permanganometric, one each of HPLC and UPLC methods were developed for pioglitazone hydrochloride (PGH). The UV-spectrophotometric method is simple, sensitive and stability-indicating and this is the first ever reported method. Two visible spectrophotometric methods using permanganate are superior to the reported spectrophotometric methods in terms of simplicity, linear range and sensitivity. The UPLC method with an LOD of 0.01 µg mL\(^{-1}\) is the most sensitive of the five methods developed. For PGH, HPLC and UPLC are the first stability-indicating methods. UPLC method for PGH uses quality by design (QbD) approach which is relatively a new technique for the assay.

For nateglinide (NTG) two UV-spectrophotometric, one each of HPLC and UPLC methods were developed for the quantification in bulk drug and its tablets. The proposed UV-spectrophotometric methods are superior to the reported spectrophotometric methods in terms of linear range and stability indicating nature. A stability-indicating RP-HPLC method was developed and validated according to ICH guidelines. A UPLC method was developed for the determination of NTG in pure drug and pharmaceutical formulations by adopting QbD which is relatively new technique in the pharmaceutical field. The method using UPLC with an LOD of 0.02 µg mL\(^{-1}\) is the most sensitive of the four methods developed. This is the first stability-indicating UPLC method reported for NTG. All the four methods are characterized by wide linear dynamic ranges, and the spectrophotometric methods, though, moderately sensitive (ε value, 10\(^3\)), are the simplest methods in terms of experimental variables involved.

Two UV-spectrophotometric, one each HPLC and UPLC methods, were developed and validated for glipizide (GPZ). Besides, these methods were used to study the extent of degradation of the drug under various stress conditions as per the ICH guidelines. The developed methods do not require an internal standard unlike the reported HPLC methods. The methods are applicable over wide linear dynamic concentration ranges. The method using UPLC with an LOD of 0.01 µg mL\(^{-1}\) is the most sensitive of the four methods developed. Both UV-
spectrophotometric and UPLC methods are the first stability-indicating methods reported so far for GPZ.

For rosiglitazone (ROS), two UV-spectrophotometric methods, one each of HPLC and UPLC methods were developed and validated. Two stability-indicating UV-spectrophotometric methods based on the measurement of absorbance of ROS solution either in 0.1M HCl or 0.1M NaOH, both at 316 nm were developed. HPLC and UPLC methods also figure in the techniques used by the author for the assay of ROS. The method using UPLC, the first stability-indicating method with an LOD of 0.01 µg mL\(^{-1}\), is the most sensitive of the four methods developed; and the method was successfully applied to spiked human urine with good recovery. All the four methods are characterized by wide linear dynamic ranges, and although the spectrophotometric methods are moderately sensitive (\(\varepsilon\) value, \(10^3\)), they are the simplest methods in terms of experimental variables involved. The proposed methods have additional advantages of simplicity of operations, are extraction-free, and are also free from heating/cooling steps and have less analysis run time.

For miglitol (MGL), one each of HPLC and UPLC methods were developed. The HPLC method is simple with short run time and is linear over the concentration range 1-750 µg mL\(^{-1}\). Besides, the methods were used to study the degradation of the drug under various stress conditions as per the ICH guidelines. The developed UPLC method is the first stability-indicating method. With an LOD of 0.003 µg mL\(^{-1}\), it is the more sensitive of the two methods developed. Both the methods are characterized by wide linear dynamic ranges; and the HPLC method has an additional advantage which can be applied to spiked human urine with satisfactory recovery.

Two chromatographic methods, HPLC and UPLC, were developed for chlorpropamide (CLP). Both methods are sensitive and stability-indicating. The developed UPLC method is the first stability-indicating method for CLP. Besides, the methods are characterized by wide linear dynamic ranges and the HPLC method has an additional advantage since it was applied to spiked human urine with good recovery. The method using UPLC with an LOD of 0.006 µg mL\(^{-1}\) is
the more sensitive of the two methods developed. A short retention time of 2.76 min enables rapid determination of the drug.

In summary, one titrimetric, twelve visible spectrophotometric, nine UV-spectrophotometric, six HPLC and eight UPLC methods were developed and validated for the determination of RPG, MFH, PGH, NTG, GPZ, ROS, MGL and CLP in pure drug form as well as in their respective formulations. For all the developed methods, the reaction products were not isolated and the reaction scheme is tentative and proposed based on the literature knowledge as well as the reactivity of the functional groups present in the drugs and based on the experimentally found reaction stoichiometry. The advantages of proposed methods over the existing methods are documented in the form of a table at the end of each chapter. One of the most important criteria i.e., the selectivity of all proposed method was tested by placebo blank and synthetic mixture analyses. Developed methods were validated as per the current requirements of the International Conference on Harmonization Guidelines (ICH Q2) which is the final word for validations all over the world. Method selectivity and accuracy were further confirmed by standard addition technique. All the methods were compared statistically with the reference method using Student’s t-test and variance ratio F-test. Some spectrophotometric methods were applicable to tablets and spiked human urine with satisfactory recovery, further confirming the high selectivity of the methods developed.

The findings of the studies are illustrated with the help of 130 figures/graphs and 6 reaction pathways/ schemes, and also supported by the data in 217 tables with 652 references. The work incorporated in the thesis offers a wide choice of methods for the assay of eight anti-diabetic drugs investigated.