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This thesis is aimed at estimation of some active pharmaceutical ingredients in pharmaceutical formulations, related substances and in biological fluids by using HPLC technique with UV detector. The author has selected four drugs for the study work. They are Linezolid (an antibiotic), Dapiprazole hydrochloride (ophthalmic solution), Pemetrexed disodium (anti cancer) and Brinzolamide (ophthalmic solution). The proficient research work has been divided into five chapters.

Chapter-1 begins with a brief account of introduction to different analytical techniques used for the drug analysis. A detailed description of high performance liquid chromatography and validation parameters was given. In this the author has also addressed the different types of impurities and pharmacokinetic techniques and its parameters.

Chapter II is divided into three parts, part-A consists of estimation of Linezolid in bulk drug and in pharmaceutical formulations and the results are as follows. The retention time (RT) was found to be 3.275 min for Linezolid. A good linear relationship \( r^2 = 0.9999 \) was observed between the concentration range of 20-60 \( \mu \text{g/mL} \). The assay of Linezolid tablets was found to be 99.944%. From the recovery studies it was found that 99.97 % was recovery of Linezolid.

Chapter II: Part-B is with regard to simultaneous determination of Linezolid and its two related substances and forced degradation study of Linezolid bulk drug. The RTs were 4.68, 21.487 and 6.232 for impurity-A, impurity-B and Linezolid respectively.
with resolution > 2. The precision was conducted using the concentration of
0.00075mg/mL of impurities and 0.5mg/mL of linezolid. The linearity concentration
range was found to be 0.000375 to 0.001125 mg/mL. The linear relationship was found
to be 0.9999. The % recovery was found to be 102%. LOD and LOQ values are
0.00174% and 0.00528% for impurity-A and 0.00226% and 0.00685% for impurity-B.
Stability study of Linezolid bulk drug under 48hrs acid sress conditions, 35.506% was
degraded. For base stress conditions it is 100%, peroxide conditions it is 59.751% and
degradation was negligible for light and heat stress conditions.

Chapter II: Part-C is related to the determination of Linezolid in human
plasma of feeding mothers. Pharmacokinetic study was conducted for twelve feeding
mothers after oral administration of one Linezolid tablet (600 mg). Efavirenz was taken
as an internal standard (IS) for this analysis. Plasma sample was prepared by LLE
method. The RT is 2.75 for Linezolid and 4.45 for IS. Linearity concentration range was
found to be 50-100µg/mL. Correlation coefficient is 0.9997, slope 0.037 intercept
0.0176 for Linezolid. % recovery was 99-102 for stability studies. Pharmacokinetics of
Linezolid was studied through non compartmental mode and C max is found to be 56.3
and Tmax is 180 min.

Under Chapter-III: part-A describes determination of Dapiprazole
hydrochloride in bulk forms is contemplated. The results are as follows. The retention
time was 3.725 min. The detector response was linear in the concentration range of 20-
120µg/mL. The respective linear regression equation being y = 1956.4x + 3409. The limit
of detection and limit of quantification was 0.5µg/mL and 1.6µg/mL respectively. The % assay of Dapiprazole hydrochloride in bulk form was 99.94%.

Chapter III, the results of simultaneous determination of Dapiprazole hydrochloride and its process related impurity-A were presented under part-B. The RTs were 16.849 and 12.422 for Dapiprazole and impurity-A respectively with resolution > 7. The precision was conducted using the concentration of 3.0µg/mL for impurity and drug respectively. The linearity concentration range was found to be 0.75-6.0µg/mL. The linear relationship was found to be 0.9998 for drug and 1.0000 for impurity. The recovery was found to be 99.90 to 103.52%. LOD and LOQ values are 1.9% and 6.4% for impurity-A. Stability study of Dapiprazole bulk drug under 48 hrs acid stress conditions, 0.08% was degraded. For base stress conditions it is 0.04%, peroxide conditions it is 8% and degradation was negligible for light and heat stress conditions.

Chapter-IV: part-A is devoted for the determination of Pemetrexed disodium in bulk and pharmaceutical formulations by gradient mode. The retention time was found to be 8.24 min. Precision of method was ascertained by using 1000µg/mL. A good linear relationship ($r^2 = 0.9998$) was observed between the concentration range of 500-1500 µg/mL. The assay of Pemetrexed injection (Prex) was found to be 99.957%. From the recovery studies it was found that about 99.877 % an average of Pemetrexed was recovered which indicates accuracy of the method. The LOD and LOQ were found to be 2.28µg/mL and 6.90µg/mL respectively.

While Chapter IV: Part-B records the results of simultaneous determination of Pemetrexed disodium and its three process related impurities. The RTs were 3.67, 8.69,
7.65 and 12.70 for Pemetrexed, impurity-A, B and C respectively with resolution > 5. The precision was conducted using the concentration of 70µg/mL and 100µg/mL for three impurities and drug respectively. The linearity concentration range was found to be 40 to 80µg/mL. The linear relationship was found to be 0.9999 for Pemetrexed and impurities A &C. For impurity C it was found to be 0.9992. The recovery was found to be 99.18 to 101.14%. LOD and LOQ values are 0.2µg/mL and 0.6µg/mL for all three impurities. Stability study of Pemetrexed disodium bulk drug under 48 hrs acid stress conditions, 30% was degraded. For base stress conditions it is 35%, peroxide conditions it is 28% and degradation for light and heat stress conditions were found to be 38%.

Under **Chapter IV: Part-C** results of determination of Pemetrexed disodium in rat plasma were presented. Pharmacokinetic study was conducted for twelve albino wistar rats by intravenous administration of Prex injection. Lopinavir was taken as an internal standard (IS) for this analysis. Plasma sample was prepared by LLE method. The RT was 3.95 for Pemetrexed and 6.6 for IS. Linearity concentration range was found to be 60-210µg/mL. Correlation coefficient was 0.9993, slope 0.016 and intercept 0.0082 for Pemetrexed in plasma. % Recovery was 98.8 to 99.87% for stability studies. Pharmacokinetics of Pemetrexed disodium was studied through non compartmental mode and C max was found to be 56.39 µg and Tmax is10 min.

**Chapter-V: part-A**describes the findings of the author in case of determination of Brinzolamide in bulk and pharmaceutical formulations. The retention time was found to be 3.77 min. Precision of method was ascertained by using 6µg/mL. A good linear relationship \( r^2 = 0.9999 \) was observed between the concentration range
of 3-9 µg/mL. The assay of Brinzolamide ophthalmic suspension (Azopt) was found to be 99.57%. From the recovery studies it was found that 99.66 % to 100.87%. The LOD and LOQ were found to be 0.33µg/mL and 1µg/mL respectively.

Chapter V: Part-B is aimed at the simultaneous determination of Brinzolamide and its two process related impurities B & C. The RTs were 3.5, 8.1 and 7.04 for Brinzolamide, impurity- B and C respectively with resolution > 2. The precision was conducted using the concentration of 8µg/mL and 20µg/mL for two impurities and drug respectively. The linearity concentration range was found to be 2 to 12µg/mL. The linear relationship was found to be 0.9949 for Brinzolamide and for impurity B & C were found to be 0.9953 and 0.9953 respectively. The recovery was found to be 99.72% to 101.24%. LOD and LOQ values are 0.006µg/mL and 0.02µg/mL for impurities B & C. Stability study of Pemetrexed disodium bulk drug under 48 hrs acid stress conditions, 5% was degraded. For base stress conditions it is 81%, peroxide conditions it is 100% and degradation for light stress condition was found to be 76%.For thermal condition degradation was negligible.

Under Chapter V: Part-C the determination of Brinzolamide in rabbit ocular fluids is contemplated. The results are as follows. Pharmacokinetic study was conducted for twelve Rabbits by intraocular administration of Azopt eye drops. Amlodipine was taken as an internal standard (IS) for this analysis. Plasma sample was prepared by LLE method. The RT was 7.5 for Brinzolamide and 5.49 for IS. Linearity concentration range was found to be 2-12µg/mL. Correlation coefficient was 0.9999, slope 0.022 and intercept 0.0542 for Brinzolamide. % Recovery was 98.66 to 101% for accuracy.
studies. Pharmacokinetics of Pemetrexed disodium was studied through non-compartmental mode and C max was found to be 6.4 µg and Tmax was 30 min.

I conclude that my thesis work in drug analysis is useful to the pharmaceutical industry which reduces the cost and wastage of chemicals due to short run times and using HPLC with simple detector like UV detector for this project. Pharmacokinetic analysis is useful to the society to know the absorption, dose proportionality and characteristics of a drug because they are life saving.