Chapter 2

Review of Literature
CHAPTER 2
LITERATURE REVIEW

2.1 TETRA SUBSTITUTED THIOPHENES

1. Tetra substituted thiophenes as anti-inflammatory agents: exploitation of analogue-based drug design.

Pillai AD, Rathod PD, Xavier FP, Padh H, Sudarsanam V, Vasu KK.

Abstract

A series of new tetrasubstituted thiophenes (4a-4i, 5a-5i and 6a-6f) have been synthesized as novel anti-inflammatory agents and were evaluated for their anti-inflammatory activity in carrageenin-induced rat hind paw oedema model at the doses of 10, 20 and 40mg/kg body weight. Among ester series, the best compound 4c showed 71% protection at 10mg/kg, 72% at 20mg/kg, and 76% at 40mg/kg to inflamed paw; while in acid series 5a showed 79% protection at 10mg/kg, 80% at 20mg/kg, and 70% at 40mg/kg, and 5c showed 72% protection at 10mg/kg, 75% at 20mg/kg, and 69% at 40mg/kg, to inflamed paw. In case of oxime series 6a-6f, the anti-inflammatory activities of the candidates were found to be poor as compared to acid and ester series. It was found on the basis of SAR studies of target compounds, that the presence of OCH(3) at R(2) position and H, OCH(3) at R(1) are one of the requirements for eliciting comparable anti-inflammatory activity in both tetrasubstituted thiophenes' ester and acid series. Compounds 4a-4i, 5a-5i were investigated for their analgesic activity in acetic acid induced writhing response model at 10mg/kg dose. Among the ester series compound 4e showed maximum protection of 60%, while 4a, 4b, and 4i exhibited 55%, 45%, and 43% protection, respectively. The result showed that presence of H, Cl at R(1) and OCH(3), CH(3) at R(2) in tetrasubstituted thiophene ester series enhances their analgesic activity. The candidates of acid series 5a-5i showed poor analgesic activity as compared to the standard drug ibuprofen. Compounds 4a-4i, 5a-5i were evaluated for their in vitro antioxidant nitric oxide radical scavenging assay. Among the ester series 4a showed maximum in vitro nitric oxide radical scavenging activity having IC(50) value 30.08microg/ml while in acid series 5a has IC(50) value 25.20microg/ml. The results
showed that the presence of R(1)=H, R(2)=OCH(3) and R(1)=R(2)=OCH(3) enhances nitric oxide radical scavenging property in tetrasubstituted thiophenes' acid series.


Molvi KI, Vasu KK, Yerande SG, Sudarsanam V, Haque N.

Abstract
A series of new tetrasubstituted thiophenes (4a-4i, 5a-5i and 6a-6f) have been synthesized as novel anti-inflammatory agents and were evaluated for their anti-inflammatory activity in carrageenin-induced rat hind paw oedema model at the doses of 10, 20 and 40mg/kg body weight. Among ester series, the best compound 4c showed 71% protection at 10mg/kg, 72% at 20mg/kg, and 76% at 40mg/kg to inflamed paw; while in acid series 5a showed 79% protection at 10mg/kg, 80% at 20mg/kg, and 70% at 40mg/kg, and 5c showed 72% protection at 10mg/kg, 75% at 20mg/kg, and 69% at 40mg/kg, to inflamed paw. In case of oxime series 6a-6f, the anti-inflammatory activities of the candidates were found to be poor as compared to acid and ester series. It was found on the basis of SAR studies of target compounds, that the presence of OCH(3) at R(2) position and H, OCH(3) at R(1) are one of the requirements for eliciting comparable anti-inflammatory activity in both tetrasubstituted thiophenes' ester and acid series. Compounds 4a-4i, 5a-5i were investigated for their analgesic activity in acetic acid induced writhing response model at 10mg/kg dose. Among the ester series compound 4e showed maximum protection of 60%, while 4a, 4b, and 4i exhibited 55%, 45%, and 43% protection, respectively. The result showed that presence of H, Cl at R(1) and OCH(3), CH(3) at R(2) in tetrasubstituted thiophene ester series enhances their analgesic activity. The candidates of acid series 5a-5i showed poor analgesic activity as compared to the standard drug ibuprofen. Compounds 4a-4i, 5a-5i were evaluated for their in vitro antioxidant nitric oxide radical scavenging assay. Among the ester series 4a showed maximum in vitro nitric oxide radical scavenging activity having IC(50) value 30.08μg/ml while in acid series 5a has IC(50) value 25.20μg/ml. The results showed that the presence of R(1)=H, R(2)=OCH(3) and R(1)=R(2)=OCH(3) enhances nitric oxide radical scavenging property in tetrasubstituted thiophenes' acid series.
   Molvi KI, Sudarsanam V, Patel MM, Haque N.

Abstract
A new series of tetrasubstituted thiophene analogues (4a-4f, 5a-5f and 8a-8i) were designed incorporating the pharmacophoric features of COX-1 (as in fenamates), 5-LOX and the p38 MAP kinase inhibitors. The designed series was synthesized by nucleophilic addition of aryl/arylisothiocyanate and enamine (2) yielding the addition product 1-(alpha-Carbomethoxy-beta-aminothiocrotonyl)-aryloaryl amines (3/7); which on reaction with substituted phenacyl bromides gave the targeted tetrasubstituted thiophene esters (4a-4f / 8a-8i). The tetrasubstituted thiophenes esters (4a-4f ) on hydrolysis with one equivalent of potassium hydroxide solution in methanol at room temperature gave corresponding acids (5a-5f ). All the targeted compounds were evaluated for their anti-inflammatory activity in carrageenin-induced rat hind paw oedema model at the doses of 10, 20 and 40 mg/kg body weight using standard drugs mefanamic acid and ibuprofen. The compounds (4c, 4e, 4f, 5f, 8a-8i) which gave reasonable protection to the inflamed paw, eliciting good or moderate comparable anti-inflammatory activity were selected for investigating their analgesic activity using acetic acid induced writhing response test in albino mice at 10 mg/kg dose using standard drug ibuprofen and in order to arrive at possible mechanism of their anti-inflammatory activity, in vitro antioxidant nitric oxide radical scavenging assay at the concentrations of 5, 10, 15, 20, 25, 30 and 35 microg/mL were performed using standard drug ascorbic acid.

   Satyendra D, Shamanna M, Janardhan S, Manoj K, Apurba T, Bhargab S, Biplab K, Rama S.

Abstract
Aim: The present study was aimed to synthesize a series of 2-substituted benzylidine imino-3-(3-chloro- 4-fluorophenyl)-carboxamido-4,5-trimethylene thiophenes SPJ-
1(a-m) and to evaluate their in-vitro anti-inflammatory activity.

Materials and Methods: The starting material (SPJ-1) was prepared by the application of versatile Gewald reaction. The In-vitro anti-inflammatory activity of synthesized compounds [SPJ-1(a-m)] was evaluated using inhibition of bovine serum albumin denaturation method.

Result: SPJ-1b and SPJ-1g have shown significant in-vitro anti-inflammatory activity.

Conclusion: The findings of the present study clearly demonstrate that chloro functional group possess inhibition of bovine serum albumin denaturation capacity and has in-vitro anti-inflammatory activity. However hydroxyl, nitro, methyl, methoxy and dimethyl amino derivatives did not show any in-vitro antiinflammatory activity.

2.2 HPLC METHOD DEVELOPMENT AND VALIDATION

   Basha SM, Rao GD, Babu CM, Rao PB, Prasad CH, Praveen PS.

Abstract
A simple, specific, accurate reverse phase liquid chromatographic method was developed for the determination of Nabumetone in bulk and in the tablet dosage forms. A waters C-18, 5μm column having 250mmX4.6mm i.d., with mobile phase containing acetonitrile:triple distilled water (70:30v/v) was used. The retention time of Nabumetone was 5.58min. The linearity for Nabumetone was in the range of 50-150μg/ml. The recovery was found to be in the range of 99.4-100.3%. The LOD and LOQ were found to be 0.1628 μg/ml, respectively. The proposed method was validated and successfully applied to the estimation of Nabumatone in the tablet formulations.

2. Determination of mosapride and Pantoprazole in a fixed-dose combination by Uv spectrophotometric methods and RP-HPLC.
   Birajdar AS, Meyyanathan SN, Suresh B.

Abstract
An accurate and reproducible UV-spectrophotometric methods and liquid
chromatographic assay method were developed and validated for the determination of mosapride and pantaprazole in capsule formulation. Two wavelengths were selected for each UV method, first simultaneous equation 274 nm, 288.2 nm and second Q value analysis method 274 nm, 302 nm was the isobestic point for both the drugs. The 30 mM ammonium sulphate buffer : acetonitrile (50:50, v/v) was used for reverse-phase liquid chromatography to determine the contents of mosapride and pantaprazole in combination-capsule dosage form. The UV and HPLC methods were validated by determining parameters such as specificity, linearity, LOD and LOQ, precision, accuracy, ruggedness and robustness. The methods were found to be specific against placebo interference. Linearity was evaluated over the concentration range of 5-50.0 μg/mL by UV and 0.5 to 5.0 μg/mL by HPLC method respectively, for mosapride and pantaprazole (the value of R2 0.999 found were by both the methods for mosapride and pantaprazole). Both the intraday and interday precision values of the systems and methods were determined. The accuracy of the methods ranged from 99.99 to 102.24 % for mosapride and from 100.45 to 101.22 % for pantaprazole. The proposed methods were found to be robust when slight but deliberate changes were made in analytical conditions. The developed methods were found suitable for the simultaneous estimation of mosapride and pantaprazole in capsule formulation as well in raw materials for quality control.

3. Method development and Validation for the Simultaneous estimation of Ofloxacin and ornidazole in Tablet dosage form by RP-HPLC.

Dhandapani B, Thirumoorthy N, Shaik H R, Rama M, Anjaneyalu N.

Abstract

A simple reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of Ofloxacin and Ornidazole in combination. The separation was carried out using a mobile phase consisting of 2mM phosphate buffer and Acetonitrile with pH 3.5 adjusted with ortho phosphoric acid in the ratio of 70: 30% v/v. The column used was Phenomenex C18, (250 mm x 4.6 mm i.d, 5μm) with flow rate of 1 ml / min using PDA detection at 293 nm. The described method was linear over a concentration range of 5-50 µg/ml and 12.5-125 µg/ml for the assay of Ofloxacin and Ornidazole respectively. Gatifloxacin (50 µg/ml)
was used as internal standard. The retention times of Ofloxacin, Ornidazole and Gatifloxacin were found to be 2.1, 2.5 and 5.5 min respectively. Results of analysis were validated statistically and by recovery studies. The limit of detection (LOD) and the limit of quantification (LOQ) for Ofloxacin and Ornidazole were found to be 5 and 10 μg/ml 10 and 25 μg/ml respectively. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Ofloxacin and Ornidazole bulk drug and in its pharmaceutical dosage form.

   Dharuman J, Vasudevan M, Somasekaran KN, Dhandapania B, Ghodea PD, Thiagarajana M.

Abstract
A simple Reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of Ofloxacin and Tinidazole in combination. The separation was carried out using a mobile phase consisting of 0.5% v/v Triethylamine buffer of pH 3.0 and Acetonitrile in the ratio of 73: 27. The column used was Kromasil C8, 5μ, 15 cm × 4.6 mm id with flow rate of 1.2 ml / min using PDA detection at 303 nm. The described method was linear over a concentration range of 10-50 μg/ml and 30-150 μg/ml for the assay of Ofloxacin and Tinidazole respectively. Ambroxol (50 μg/ml) was used as internal standard. The retention times of Ofloxacin, Tinidazole and Ambroxol were found to be 2.3, 4.1 and 5.1 min respectively. Results of analysis were validated statistically and by recovery studies. The limit of quantification (LOQ) for Ofloxacin and Tinidazole were found to be 10 and 30 μg/ml respectively. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Ofloxacin and Tinidazole bulk drug and in its pharmaceutical dosage form.
5. High Performance Liquid Chromatographic Method For Determination of Amoxicillin trihydrate and bromhexine hydrochloride in oral dosage forms.
Dhoka M, Gawande V, Joshi P.

Abstract
A simple high performance liquid chromatographic method is reported for the simultaneous determination of Amoxicillin Trihydrate and Bromhexine Hydrochloride in oral dosage forms. Investigated drugs were resolved on HiQ Sil C18 (4.6x250mm, 5µm) reverse-phase column, utilizing a mobile phase of Methanol:0.02M Ammonium acetate, pH5 (adjusted with orthophosphoric acid 10% aqueous) 90:10v/v. Mobile phase was delivered at the flow rate of 1.0mL/minute. Ultra violet detection was carried out at 254nm. Separation was completed within 10 minutes. Calibration curves were linear with correlation coefficient 0.993 and 0.995 over a concentration range of 100-300µg/ml for Amoxicillin Trihydrate and 2-10 µg/ml for Bromhexine Hydrochloride respectively. Recovery was between 99.5-101.32 percent and 98.93-101.18 percent for Amoxicillin Trihydrate and Bromhexine Hydrochloride respectively. Method was found to be reproducible with relative standard deviation(R.S.D.) for intra and interday precision to be <1.5% over the said concentration range.

Gandhimathi M, Saravanakumar M, Ravi TK.

Abstract
A simple, sensitive and rapid ionpair high performance liquid chromatographic method was developed for the estimation ceftriaxone sodium (CS) and tazobactum sodium (TS) in pharmaceutical dosage forms. LichrocartR100-RP18e5)m-C18 column was used with a mobile phase containing mixture of 0.012M tetra butyl ammonium hydroxide in 0.01M potassium dihydrogen phosphate : acetonitrile in the ratio of 70:30 % v/v. The flow rate was 0.8ml/min and effluents were monitored at 220nm and eluted at 4.5 and 6.7 min for tazobactum sodium (TS) and ceftriaxone sodium (CS) respectively. Calibration curve was plotted with a range from 2 to 12 g/ml (CS) and 0.26 to 1.56 (TS) g/ml. The assay was validated for the parameters.
like accuracy, precision, robustness and system suitability parameters. The proposed method can be useful in the routine analysis for the determination of ceftriaxone sodium and tazobactum sodium in pharmaceutical dosage forms.


Godse VP, Bafana YS, Deshapande, SY, Vyas MR, Bhosale AV.

Abstract
The present paper describes development of stability-indicating RP-HPLC method for the simultaneous determination of Ofloxacin and Satranidazole in presence of its degradation products, generated from forced degradation studies. Ofloxacin and Satranidazole and their combination drug product were exposed to acid, base, neutral hydrolysis; oxidation, dry heat, photolytic stress conditions and the stressed samples were analyzed by proposed method. The proposed HPLC method utilizes HiQ sil C18W column (250mm × 4.6mm i.d., 5μm) of KYA TECH, Corporation and a mobile phase comprising of acetonitrile: phosphate buffer (pH3) in ratio of 35:65v/v with flow rate of 1ml/min. The retention time of OFLX and STZ was found to be 2.85min and 6.25min respectively. Quantitation was achieved with UV detection at 296nm for OFLX and 320nm for STZ. The method has been validated for ofloxacin and satranidazole in terms of accuracy, precision, linearity, LOD, LOQ and robustness. The developed validated stability-indicating HPLC method was found to be simple, specific, accurate and reproducible for the determination of instability of these drugs in bulk and commercial products.


Hsu MC, Hsu PW.

Abstract
A reversed-phase column liquid chromatographic method was developed for the assay of amoxicillin and its preparations. The linear calibration range was 0.2 to 2.0 mg/ml (r = 0.9998), and recoveries were generally greater than 99%. The high-performance
liquid chromatographic assay results were compared with those obtained from a microbiological assay of bulk drug substance and capsule, injection, and granule formulations containing amoxicillin and degraded amoxicillin. At the 99% confidence level, no significant inter method differences were noted for the paired results. Commercial formulations were also analyzed, and the results obtained by the proposed method closely agreed with those found by the microbiological method. The results indicated that the proposed method is a suitable substitute for the microbiological method for assays and stability studies of amoxicillin preparations.


Jian Z, Xiao-hui S, Wei W, Ke S.

Abstract

OBJECTIVE: To establish a RP-HPLC method for determination of the related substances in tiotropium bromide crude drug and the content of main component in tiotropium bromide dry powder inhalation. METHODS: The determination was performed on Phenomenex Luna chromatographic column, the mobile phase consisted of acetonitrile-2% triethylamine solution (pH 5.5, 2773) at a flow rate of 1.0 mL·min-1. The detection wavelength was set at 237 nm. RESULTS: The linear range of tiotropium bromide was 1.0–50 μg·mL-1 and the average recovery rate was 100.1% (RSD=0.7%). The total contents of the related substances in three batches of samples were all less than 0.5%. The labeled amount of the tiotropium bromide dry powder inhalation was 99.52%–100.30%. CONCLUSION: This method is proved to be simple and accurate and it is applicable for the quality control of the crude drug and the preparation of tiotropium bromide.

10. Content Determination of Oxaliplatin in Oxaliplatin Liposome by HPLC

Jing Z, Hua Z, Xin-hui J.

Abstract

OBJECTIVE: To establish an HPLC method for the content determination of oxaliplatin in Oxaliplatin liposome. METHODS: The separation was performed on
RESULTS: The linear range of oxaliplatin was 12.2–122.0 μg·mL⁻¹ (n=5, r=0.9999) with an average recovery of 98.58% (RSD=1.83%). The limit of detection (LOD) was 97.6 ng·mL⁻¹.

CONCLUSION: The method is simple, sensitive and accurate for the content determination of oxaliplatin in Oxaliplatin liposome.


Kumar R, Singh P, Singh H.

**Abstract**

A simple, sensitive, and precise high performance liquid chromatographic method for the analysis of naproxen and pantoprazole has been developed, validated and used for the determination of compounds in commercial pharmaceutical products. The compounds were well separated on a Hypersil BDS C-18 reversed-phase column by use of a mobile phase consisting of 0.1 M sodium acetate (pH 8.2), acetonitrile and methanol (70:20:10 v/v) at a flow rate of 1.0 mL min⁻¹ with detection wavelength at 285 nm. The linearity ranges were 5–70 μg mL⁻¹ for naproxen, and 5–40 μgmL⁻¹ for pantoprazole. The recovery amount was more than 99%. The high recovery and low relative standard deviation confirms the suitability of the method for determination of naproxen and pantoprazole in pharmaceutical dosage forms.


Lakka NS, Goswami N.

**Abstract**

A RP-HPLC method was developed for estimation of 5-Hydroxy methyl furfural content in Levofloxacin Hemihydrate intravenous infusion 5mg/ml. It involved a 150mm x 4.6mm, 5μm LichroSphere C-18 column with 40°C temperature. The separation was achieved on simple isocratic method. A mixture of pH 3.0 buffer (0.04M, ortho phosphoric acid buffer, adjusted pH 3.0 with triethylamine)
acetonitrile (87:13, v/v) was used as the mobile phase. The flow rate was 1.0ml/min, injection volume was 5μl and the detection wavelength was 284nm. The re-tention time of 5HMF was 2.7min. The total runtime was 12min within which active compound and degradation products were separated. The developed method successfully applied to the determination of 5HMF content in pharmaceutical preparations. The developed RP-HPLC method was validated with respect to linearity, accuracy, specificity, limit of detection, limit of quantification, precision, robustness and ruggedness.

Lalit VS, Bari SB.

Abstract
A new simple, rapid and precise reverse phase high pressure liquid chromatography (RP-HPLC) method was developed for the simultaneous estimation of amoxicillin trihydrate and bromhexine hydrochloride from oily suspension. An ODS C18 (250 X 4.5mm ID), 5 μ particle size with mobile phase methanol and glacial acetic acid (50:50 v/v) were used. The flow rate was 1.0ml/min and responses were measured at 254 nm. The retention time for amoxicillin trihydrate and bromhexine hydrochloride were observed at 3.04 and 8.18 min. respectively. Linearity for amoxicillin trihydrate and bromhexine hydrochloride were in the range of 8-50 mcg/ml and 5-25 mcg/mL respectively. Percent recovery was 99.54% and 98.65% for amoxicillin trihydrate and bromhexine hydrochloride respectively. The proposed method can be applied for the routine analysis of amoxicillin trihydrate and bromhexine hydrochloride in combination.

Lalitha DM, Chandrasekhar KB.
Abstract
The objective of current study was to develop a validated specific stability indicating reversed-phase liquid chromatographic method for the quantitative determination of levofloxacin as well as its related substances determination in bulk samples, pharmaceutical dosage forms in the presence of degradation products and its process related impurities. Forced degradation studies were performed on bulk sample of levofloxacin as per ICH prescribed stress conditions using acid, base, oxidative, water hydrolysis, thermal stress and photolytic degradation to show the stability indicating power of the method. Significant degradation was observed during oxidative stress and the degradation product formed was identified by LCMS/MS, slight degradation in acidic stress and no degradation was observed in other stress conditions. The chromatographic method was optimized using the samples generated from forced degradation studies and the impurity spiked solution. Good resolution between the peaks corresponds to process related impurities and degradation products from the analyte were achieved on ACE C18 column using the mobile phase consists a mixture of 0.5% (v/v) triethyl amine in sodium dihydrogen orthophosphate dihydrate (25 mM; pH 6.0) and methanol using a simple linear gradient. The detection was carried out at 294 nm. The limit of detection and the limit of quantitation for the levofloxacin and its process related impurities were established. The stressed test solutions were assayed against the qualified working standard of levofloxacin and the mass balance in each case was in between 99.4 and 99.8% indicating that the developed LC method was stability indicating. Validation of the developed LC method was carried out as per ICH requirements. The developed LC method was found to be suitable to check the quality of bulk samples of levofloxacin at the time of batch release and also during its stability studies (long term and accelerated stability).

Nikam DS, Bonde CG, Surana SJ, Venkateshwarlu G, Dekate PG.

Abstract
A reverse phase high performance liquid chromatographic method was developed for
simultaneous determination of amoxicillin trihydrate and flucloxacillin sodium in bulk and pharmaceutical formulation. The separation was made by a Kromasil C18 column (250 cm x 4.6 mm, 5μm) using 0.020 M potassium dihydrogen orthophosphate - acetonitrile (75:25) as mobile phase. The validation of the method was performed, and specificity, reproducibility, precision and accuracy were confirmed. The limits of quantification were approximately 0.16 μg/ml for amoxicillin trihydrate and 0.25 μg/ml for flucloxacillin sodium. Due to simplicity and accuracy the method particularly suitable for routine pharmaceutical quality control.

   Palanikumar B, Thenmozhi A, Sridharan D

Abstract
An isocratic liquid chromatographic method with UV detection at 230nm is described for simultaneous determination of ceftriaxone sodium and sulbactam sodium in ceftriax-s 1.5gm injection. Chromatographic separations of two drugs was achieved on a Hypersil ODS C-18 column (250mmX4.6mm, i.d., 5 μm) using a mobile phase consisting of 10mM Potassium Dihydrogen Orthophosphate and acetonitrile (90:10%v/v) adjusted to pH 5.0 with Potassium hydroxide, in the flow rate of 1.0mL/min. The optimum separation was achieved in less than 15minutes. The developed liquid chromatographic method offers symmetric peak shape, good resolution and reasonable retention time for both drugs. The method was validated as per ICH guidelines. The developed method obeys beer’s law over the concentration range of 140-250μg/ml for Ceftriaxone and 75 to 160 μg/ml for sulbactam sodium.

   Rao BM, Chakraborty A, Srinivasu MK, Devi ML, Kumar PR, Chandrasekhar KB, Srinivasan AK, Prasad AS, Ramanatham J.

Abstract
A novel stability-indicating high-performance liquid chromatographic assay method was developed and validated for docetaxel in the presence of degradation products generated from forced decomposition studies. A gradient HPLC method was
developed to separate the drug from the degradation products, using a Hichrom RPB HPLC column. Mixture of water and acetonitrile was used as mobile phase. The flow rate was 1.0 ml/min and the detection was done at 230 nm. Using the above method one can carry out the quantitative estimation of impurity namely DCT-1 and docetaxel. The developed gradient LC method was subsequently validated.

   Rao S, Mastanamma SK, Prahlad P, Anantha kumar D.

Abstract
A rapid and reproducible reverse phase high performance liquid chromatographic method has been proposed for the estimation of docetaxel in its pure form as well as in injectable dosage forms. Chromatography was carried out on a BDS C8 column (150 x 4.6mm; 5μ) using a mobile phase composed of methanol - water (70:30 v/v), which was pumped at a flow rate of 1.5ml/min. The detection of the drug was monitored at 232 nm. Paclitaxel was used as an internal standard. The retention time of the drug was found to be 7.4 min. The method produced linear responses in the concentration range of 0.1 to 60 g/mL of docetaxel.

   Reddy AM, Banda N, Shinde GD, Rao DV, Kocherlakota CS, Krishnamurthy V.

Abstract
New stability indicating chromatographic methods have been developed for estimation of Assay and Impurities of Docetaxel in Docetaxel injection for evaluation of pharmaceutical quality. With this method, the process related impurities and degradants are well separated from the peaks due to placebo. The relative retention times and relative response factors of the known impurities have been established. The LOQ of the known impurities and docetaxel are found to be less than 0.2 μg /ml and the recovery falls in the range of 90–110%. Peak purities demonstrated the stability indicating nature of the methods. The methods developed in the present study
overcome the lacunae of the existing published methodologies in evaluation of the quality of Docetaxel injection. In essence, the present study provides an improved methodology for evaluation of the pharmaceutical quality of Docetaxel injection.

Reddy BP, Reddy R, Ramachandran D.

Abstract
A simple, sensitive and precise high performance liquid chromatographic method for the analysis of pantoprazole sodium and lansoprazole has been developed, validated and used for the determination of compounds in commercial pharmaceutical products. The compounds were well separated isocratically on a C18 column [Inertsil C18, 5 μ, 150 mm x 4.6 mm] utilizing a mobile phase consisting of acetonitrile: phosphate buffer (60:40, v/v, pH 7.0) at a flow rate of 1.0 mL/min with UV detection at 230 nm. The retention time of pantoprazole sodium and lansoprazole was found to be 2.017 min and 2.538. The procedure was validated for linearity (Correlation coefficient=0.999). The study showed that reversed-phase liquid chromatography is sensitive and selective for the determination of pantoprazole sodium and lansoprazole using single mobile phase.

21. Determination of Pantoprazole sodium and Lansoprazole in individual dosage form tablets by RPHPLC Using single mobile phase.
Reddy P, Jayaprakash M, Kotta S.

Abstract
A simple, sensitive and precise high performance liquid chromatographic method for the analysis of Pantoprazole sodium and lansoprazole has been developed, validated and used for the determination of compounds in commercial pharmaceutical products. The compounds were well separated isocratically on a C18 column [Use Inertsil C18, 5 μ, 150 mm x 4.6 mm] utilizing a mobile phase consisting of acetonitrile: phosphate buffer (60:40, v/v, pH 7.0) at a flow rate of 1.0 ml/min with UV detection at 230 nm. The retention time of Pantoprazole sodium and lansoprazole was found to be 2.017 min and 2.538. The procedure was validated for linearity
(Correlation coefficient = 0.999). The study showed that reversed-phase liquid chromatography is sensitive and selective for the determination of Pantoprazole sodium and lansoprazole using single mobile phase.

22. Analysis of Nabumetone in Bulk and Tablet Formulation by a New and Validated Reverse Phase High Performance Liquid Chromatography.
Sahu PK, Annapurna MM.

Abstract
RP-HPLC analytical method for the estimation of nabumetone in pharmaceutical dosage forms was developed and validated. A Hypersil ODS C18, 4.6 mm x 250 mm, 5 μm column from Supelco (India), with mobile phase comprised of acetonitrile: triple distilled water (50:50) with a total run time of 18 min was used and the wavelength of the detector was set at 230 nm. Stavudin is used as internal standard. The retention times were 14.167 min and 1.967 min for nabumetone and stavudin (IS) respectively. The extraction recovery of nabumetone from pharmaceutical dosage form (tablets) was >101% and the calibration curve was linear (r² = 0.995) over nabumetone concentrations ranging from 1 to 200 μg/mL. The method had an accuracy of >99% and LOD and LOQ of 0.17482 μg/mL and 0.5827 μg/mL respectively. The method reported is simple, reliable, precise and accurate and has the capability of being used for determination of nabumetone in bulk and pharmaceutical dosage forms.

Venishetty VK, Parikh N, Sistla R, Ahmed FJ, Diwan PV.

Abstract
Docetaxel has significant single agent activity in prostate cancer and ketoconazole also has activity as a second line hormonal agent. In vitro, ketoconazole is synergistic with some chemotherapy agents by enhancing the intracellular retention of the cytotoxic agent. A potential drug-drug interaction exists though between docetaxel and ketoconazole because both agents are metabolized hepatically by the cytochrome P-450 system. Hence, a nanoparticulate system was formulated by loading both drugs
for tumor targeting. Assay and in vitro release of the formulation were conducted by developing simple, precise, accurate, and validated analytical method for simultaneous determination docetaxel and ketoconazole using reversed-phase high-performance liquid chromatography (RP-HPLC). The RP-HPLC method was developed using Waters Symmetry C(18) column (25 cm — 4.5 mm, 5 μm) with a mobile phase consisting of acetonitrile and 0.2% triethylamine pH adjusted to 6.4 (48:52, v/v) at flow rate of 1 mL/min. Intra-day and inter-day variations were less than 2% over the linearity range, 0.5-20 μg/mL. The proposed two methods were successfully applied for the determination of docetaxel and ketoconazole in solid lipid nanoparticles.

24. Determination of Tiotropium Bromide by HPLC.
Yan L, Zheng-Min M.

Abstract
A HPLC method was established for the determination of tiotropium bromide. A C18 column was used with the mobile phase of methanol -50mmol/L sodium acetate buffer solution (500–500, containing 1% triethylamine, adjusted pH to 4.0 by phosphoric acid) at the detection wavelength of 236nm. The calibration curve was linear in the range of 6 - 14μg/ml. The average recovery was 99.5% with RSD of 0.62%.

25. HPLC determination of levoisomer of oxaliplatin.
Yu BL, Yang L, Ma YP.

Abstract
Objective: To develop an effective method for determination of levoisomer of oxaliplatin by HPLC. Methods: Two portions of quantitative racemic oxaliplatin were weighed, one was dissolved by deionized water, the other was dissolved by mobile phase. Both of concentration were 1.0 mg mL⁻¹. Two portions of quantitative oxaliplatin were weighed, one was dissolved by deionized water, the other was dissolved by mobile phase. Both of concentration were also 1.0 mg mL⁻¹. Chromatographic condition: chiral chromatographic column was Chiralcel OC (250 mm, 4.6 mm, 10 μm). The column contains a carbamate cellulose derivative
coated silica-gel as the packing material of Daicel chemical industry company of Japan; The mobile phase was methanol-ethanol (70 : 30); The flow rate was 0.5 mL min⁻¹; The detector was set at 250 nm; Column temperature was room temperature; Injection volume was 20 µL. Results: The resolution was 3.4, the number of theoretical plates was 6498 and the peak form was good and there was no reversal peak when the sample was dissolved by the mobile phase. The result was consistent with the regulation of quality criteria of oxaliplatin. However, the resolution was 1.4, the number of theoretical plates was 1246 and the peak form was not good and there was reversal peak when the sample was dissolved by deionized water. The result was not consistent with the regulation of quality criteria of oxaliplatin. Conclusions: An effective method for determination of levoisomer of oxaliplatin by HPLC is developed.

2.3 BIOANALYTICAL METHOD AND PHARMACOKINETIC STUDY

1. HPLC determination of amoxicillin comparative bioavailability in healthy volunteers after a single dose administration.
   
   Abreu LR, Ortiz RA, Castro SC, Jose PJ.

Abstract

PURPOSE: An accurate, precise and sensitive HPLC assay was developed for the determination of amoxicillin in human plasma samples, to compare the bioavailability of two amoxicillin capsule (500mg) formulations (Amoxicilina from Brazil, as a test formulation and Amoxil® from SmithKline Beecham Laboratories Ltda., Brazil, as a reference formulations) in 24 volunteers of both sexes. METHODS: Amoxicillin concentrations were analyzed by combined reversed phase liquid chromatography and UV detection (λ=229 nm). Amoxicillin and cefadroxil (internal standard) were extracted from the plasma by addition of cold methanol. The separation was achieved using the Lichrosorb® 10µm, C18 reversed phase column at room temperature. The mobile phase consisted of a 95% phosphate buffer (0.01mol/L), pH=4.8 and 5% acetonitrile mixture. The study was conducted using an open randomized 2-period crossover balanced design with a 1-week washout period between the doses. Plasma
samples were obtained over an 8-hour period. The bioequivalence between the two formulations was assessed by calculating individual peak plasma concentrations (Cmax) and area under the curve (AUC0-8h) ratios (test/reference). The statistical interval proposed was 80-125%, as established by the US Food and drug administration Agency. RESULTS: The internal standard and amoxicillin eluted about 4.2 and 5.2 min, respectively at a flow rate of 1.3ml/min. The mean absolute recovery of AMO in plasma was 90.0% at 3µg/ml, 98.6% at 25µg/ml and 95.3 at 50µg/ml. The assay showed excellent relationships between peak height ratios and plasma concentrations (r2 ≥ 0.999). The limit of quantification was 1g/ml, based on 200l of plasma. The geometric mean of Amoxicilina/Amoxil® 500mg capsules individual percentage ratio was 101.4% for AUC0-8h, and 99.9% for Cmax. The 90% confidence intervals were 98.3-104.4% and 95.7-103.9%, respectively. CONCLUSION: This simple, rapid and selective method is suitable for pharmacokinetic, bioavailability and bioequivalence studies. Since the 90% CI for both Cmax and AUC0-8h lies within the 80-125% interval proposed by the Food and Drug Administration, it was concluded that Amoxicilina 500mg capsules was bioequivalent to Amoxil® capsules 500mg, in terms of both the rate and extent of absorption.

   Amini M, Khanavi M, Shafiee A.

Abstract
A rapid, simple and sensitive high-performance liquid chromatography method was developed for determination of ciprofloxacin in plasma by means of ultraviolet detection. Ofloxacin was used as an internal standard and separation carried on a Novapak C18 column using a mobile phase of 0.01 M phosphate buffer (pH =2.6): methanol (82:18 v/v). Extraction of drug was performed from plasma by liquid-liquid extraction and the average recovery was 78.2% The assay is precise, with inter-assay coefficient of variation of 6.70 % at 0.25-8 kg/ml (n=3). Using UV detection at 277 nm the detection limit for ciprofloxacin was 20 ng/ml of plasma and the mean
extraction recovery was 78.2 %. Short elution time, using UV detector and usage of ofloxacin as internal standard are advantages of this method.

3. High sensitivity assays for docetaxel and paclitaxel in plasma using solid-phase extraction and high-performance liquid chromatography with UV detection.

Andersen A, Warren DJ, Paal FS, Kristensen GB, Olsen H.

Abstract

Background: The taxanes paclitaxel and docetaxel have traditionally been used in high doses every third week in the treatment of cancer. Lately there has been a trend towards giving weekly low doses to improve the therapeutic index. This article describes the development of high performance liquid chromatographic (HPLC) methods suitable for monitoring taxane levels in patients, focusing on patients receiving low-dose therapy.

Methods: Paclitaxel and docetaxel were extracted from human plasma by solid phase extraction, and detected by absorbance at 227 nm after separation by reversed phase high performance liquid chromatography. The methods were validated and their performance were tested using samples from patients receiving paclitaxel or docetaxel. Results: The limits of quantitation were 1 nM for docetaxel and 1.2 nM for paclitaxel. For both compounds linearity was confirmed from the limit of quantitation up to 1000 nM in plasma. The recoveries ranged between 92% and 118% for docetaxel and between 76% and 104% for paclitaxel. Accuracy and precision were within international acceptance criteria, that is within ± 15%, except at the limit of quantitation where values within ± 20% are acceptable.

Low-dose patients included in an on going clinical trial had a median docetaxel concentration of 2.8 nM at 72 hours post infusion. Patients receiving 100 mg/m2 of paclitaxel had a mean paclitaxel concentration of 21 nM 48 hours after the end of infusion. Conclusion: We have developed an HPLC method using UV detection capable of quantifying 1 nM of docetaxel in plasma samples. The method should be useful for pharmacokinetic determinations at all relevant doses of docetaxel. Using a similar methodology paclitaxel can be quantified down to a concentration of 1.2 nM in plasma with acceptable accuracy and precision. We further demonstrate that the

Aqil M, Ali A, Ahad A, Sultana Y, Najmi AK, Saha N.

Abstract

A high-performance reversed-phase liquid chromatographic method for quantification of metoprolol tartrate (MT) in human plasma is presented. A C18 column was used with acetonitrile–water–triethylamine 18:81:1 (v/v) as mobile phase and pinacidil monohydrate as internal standard (IS). UV detection was at 275 nm and MT and the IS were detected at retention times of 1.5 and 2.6 min, respectively. The method is sensitive with a limit of quantification of 20 ng mL⁻¹. The calibration plot for MT in spiked plasma was linear in the concentration range 20–200 ng mL⁻¹. Within-batch and total accuracy of the method ranged between 99.71% and 101.61%, and within-batch and total precision, expressed as the coefficient of variation, was 0.20–2.13%. Recovery of MT from spiked plasma was 97.00% and the freeze–thaw and bench-top stability of samples ranged from 77.92 to 105.62%. The method can be successfully used for analysis of MT in human plasma during pharmacokinetic studies.

5. High performance thin layer chromatographic determination of ofloxacin in plasma.

Bhandari SS, Khisti NA, Gandhi SV.

Abstract

A new simple High Performance Thin Layer Chromatographic (HPTLC) method for determination of Ofloxacin in plasma has been developed and validated. A Simple precipitation method was carried out by using Trichloroacetic acid and a known amount of supernatant solution was spotted on precoated silica gel 60 F254 plates using a Camag Linomat IV autosampler. Detection and quantitation were performed without using an internal standard. The mobile phase selected was n-butanol: Methanol: Ammonia (6:1:3 v/v/v) with UV detection at 295 nm. The calibration curves of Ofloxacin in methanol and in plasma were linear in range 100-600 ng. The limit of quantization for Ofloxacin in human plasma was 100 ng and no interference
was found from endogenous compounds. The recovery of Ofloxacin from human plasma using the described precipitation procedure was about 88.1047%. The method provides a direct estimate of the amount of Ofloxacin present in human plasma.

   Carlucci G, Mazzeo P, Vetuschi C.

Abstract
The development and validation of a reversed-phase liquid chromatographic method for the determination of lomefloxacin in seminal plasma is described. Lomefloxacin was extracted on a solid-phase cartridge and separated on a reversed-phase column with acetonitrile in phosphate buffer at pH 7.0 as mobile phase. The solid-phase extraction showed high recovery for lomefloxacin from seminal plasma samples. The chromatographic peaks height ratio of lomefloxacin and internal standard, obtained by fluorimetric detection, was used for quantitative analysis. The proposed method was validated with respect to accuracy, precision, linearity, and specificity. Also, the method was determined to be robust with regards to the following parameters: mobile phase, apparent pH; mobile phase organic content. The percent recoveries of lomefloxacin, the limit of detection (LOD) and limit of quantitation (LOQ) for the HPLC method have been determined. This high-performance liquid chromatographic method has been successfully used in medical laboratories to assay seminal plasma samples for studies on the treatment of chronic bacterial infections with lomefloxacin.

   Cho SH, Im HT, Park WS, Ha YH, Choi YW, Lee KT.

Abstract
A rapid and simple high-performance liquid chromatography (HPLC) method was developed and validated for the quantification of clindamycin in human plasma. After precipitation with 50% trichloroacetic acid (TCA) containing the internal standard, propranolol, the analysis of the clindamycin level in the plasma samples was carried out using a reverse-phase cyano (CN) column with ultraviolet detection (204 nm).
The chromatographic separation was accomplished with an isocratic mobile phase consisting of acetonitrile-distilled water-7.6 mm tetramethylammonium chloride (TMA) (60:40:0.075, v/v/v), adjusted to pH 3.2. The proposed method was specific and sensitive with a lower limit of quantitation (LLOQ) of 0.2 microg/mL. This HPLC method was validated by examining the precision and accuracy for inter- and intraday analysis in the concentration range 0.2-20.0 microg/mL. The relative standard deviations (RSD) in the inter- and intraday validation were 6.1-14.9 and 6.0-16.1%, respectively. In the stability test, clindamycin was found to be stable in human plasma during the storage and assay procedure. The present HPLC method was applied to the analysis of samples taken up to 12 h after a single oral administration of clindamycin in healthy volunteers.

8. Bioequivalence study of levofloxacin tablets in healthy Indian volunteers using HPLC.
   Das A, Mukherjee J, Dey G, Sarkar AK, Sahoo BK, Chakrabarty US, Nandi U, Pal TK.

Abstract
An improved HPLC method was developed and validated for the determination of concentration of levofloxacin (CAS 100986-85-4) in human plasma. This paper is an attempt to compare the bioavailability of two levofloxacin tablet formulations (reference and test) containing 500 mg of levofloxacin. Both the formulations were administered orally as a single dose, separated by a washout period of 1 week. The HPLC method was validated by examining the precision and accuracy for the interday and intra-day runs in a linear concentration range of 0.10-10.00 microg/ml. Bioequivalence of two formulations were determined in 12 healthy, Indian, male volunteers in a single-dose, two-period, two-sequence, two-treatment crossover study. The content of levofloxacin in plasma was determined using HPLC with UV detection. The formulations were compared using the following pharmacokinetic parameters: area under the plasma concentration-time curve (AUC(o-t)), area under the plasma concentration-time curve from zero to infinity (AUC(o-infinity)), peak plasma concentration (C(max)), and time to reach peak plasma concentration (t(max)). The results indicated that there were no statistically significant differences
(P > 0.05) between the logarithmically transformed AUC(o-infinity), and C(max) values of the test and reference formulations. The 90% confidence interval for the ratio of the logarithmically transformed AUC(o-t), AUC(o-infinity) and C(max) were within the bioequivalence limit of 0.8-1.25 and the relative bioavailability of the test formulation was 99.98% of that of the reference formulation.

   Dharuman JG. Rajasekaran A, Krishnasamy K, Thiagarajan M, Somasundaram

Abstract
A rapid, sensitive and selective HPLC method for the determination of levofloxacin in rat plasma is developed and validated. Separation is done on a Phenomenex C18 RP column with a mobile phase of acetonitrile – 0.4 % triethylamine (pH 3.1) in the ratio of 18:82 % v/v and detection at 295 nm. The standard curve is linear (r > 0.998) over the concentration range of 20.0–5000 ng/ml. The lower limit of quantification (LOD) for levofloxacin is 10.0 ng/ml. The maximum concentration (Cmax) obtained for two brands Levoflox and Generic formulation are 132.72 and 113.97 ng/ml respectively. The half life (t1/2) of levofloxacin for Levoflox and Generic are calculated as 1.763 and 1.628 h. Area under the curve AUC t0 of Levoflox and Generic is calculated as 271.18 and 279.84 ng h/ml. Elimination rate constant (kel) is calculated for Levoflox and Generic from the slope of log concentration versus time curve with regression analysis. Elimination rate constant is found to be 0.393 and 0.4272 h-1. This study shows that there is no significant difference in kinetic parameters between two products. So the two formulations are considered to be bioequivalent.

10. Development and validation of a sensitive analytical method for the simultaneous determination of buprenorphine and norbuprenorphine in human plasma.
    Gopal S, Tzeng TB, Cowan A.

Abstract
A sensitive, specific, and robust capillary gas chromatography-mass spectrometry
method has been developed and validated for simultaneous determination of buprenorphine and its active metabolite, norbuprenorphine, in human plasma. Sample preparation involved a clean-up procedure using a Bond Elut Certify cartridge followed by derivatization with pentafluoropropionic anhydride. Separation was carried out on a HP-1 fused silica capillary column using helium as the carrier gas. Selected ion monitoring was used in the electron impact mode. Excellent linearity was found between 0.10 and 20.0 ng/ml with a limit of quantitation of 0.05 and 0.10 ng/ml for buprenorphine and norbuprenorphine, respectively. Interday and intraday assay precisions (%CV) and accuracies were within 15.0% for buprenorphine and norbuprenorphine, respectively. Recoveries were quantitative and concentration-independent. This method will be applied to pharmacokinetic/pharmacodynamic/bioequivalence studies of buprenorphine in humans.

11. Validation of an HPLC method for the determination of ciprofloxacin in human plasma.
   Imre S, Dogaru MT, Vari CE, Muntean T, Kelemen L.

Abstract
A simple HPLC method with fluorescence detection of ciprofloxacin in human plasma was developed and validated. After protein precipitation, chromatographic separation of ciprofloxacin in plasma was achieved at 35 degrees C with a C18 column and acetonitrile-phosphate mixture, pH 3, as mobile phase. Quantitative determination was performed by fluorimetry after excitation at 278 nm. The method was specific and validated with a limit of quantification of 41 ng/ml. The intra- and inter-day coefficients of variation were between 0.5 and 6.6% and accuracy between -2.02 and 7.04%. Ciprofloxacin was stable in plasma for 40 days at -20 degrees C and after three freezing-thawing cycles. The method has been applied in a bioequivalence study of two formulation of 500 mg ciprofloxacin.

12. Bioequivalence Evaluation of Lomefloxacin 400 mg Tablets(Lomax® Versus Maxaquin®) in Healthy Human Volunteers.
Abstract
This study represents the results of a randomized, single dose, two-treatment, two-period crossover study in 18 healthy male volunteers to assess the bioequivalence of two tablets of 400 mg lomefloxacin. The two formulations were: Lomax® (Julphar, United Arab Emirates) as the test formulation and Maxaquin® (Searle, S.A., UK) as the reference formulation. The study was conducted at the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia, jointly with King Khalid University Hospital, Riyadh, Saudi Arabia. After overnight fasting the two products were administered as a single dose on two treatment days separated by a 1 week washout period. Serial blood samples were collected thereafter, for a period of 48 h. Plasma harvested from blood was analysed for lomefloxacin by a sensitive, reproducible and accurate HPLC method. Various pharmacokinetic parameters including AUC0–t, AUC0–_, Cmax, Tmax, T1/2, Kelm and Cmax:AUC0–_ were determined from plasma concentrations for both formulations and found to be in good agreement with reported values. Statistical modules applied to AUC0–t, AUC0–_ and Cmax revealed no significant difference in the two tested products. Based on these statistical inferences it was concluded that Lomax® is bioequivalent to Maxaquin®.

13. Determination of unmetabolized folic acid in human plasma using affinity HPLC.
Kalmbach R, Paul L, Selhub J.

Abstract
Background: Folic acid (FA) fortification of food created the need to determine whether fortification elevated concentrations of unmetabolized FA in plasma and whether this form of the vitamin in blood is associated with adverse health outcomes. Objective: The objective of this research was to devise a simple, rapid method for the measurement of unmetabolized plasma FA in epidemiologic studies. Design: We previously used the affinity/HPLC with electrochemical detection method to measure folate distribution in human plasma and red blood cells (RBCs). We modified this method with the inclusion of synthetic ethyltetrahydrofolate as an internal standard and with the use of 2 affinity columns connected in parallel to the analytic column through a switching valve to allow one column to be loaded while the other column
was eluted into the analytic column. Results: We identified FA and 5-methyltetrahydrofolate (5-mTHF) by retention time and characteristic response across the channels of the electrochemical detector. Limits of detection were 0.034 pmol for 5-mTHF and 0.027 pmol for FA per injection, and the recovery was 92.2% (5-mTHF) and 98.9% (FA). CVs for samples were 8.1% (within day) and 6.8% (between day) for 5-mTHF and 3.2% (within day) and 5.9% (between day) for FA. Total folate with the use of this method correlated highly ($r^2 = 0.98, P < 0.001$) with values from the microbial assay. The run time for the method was 30 min per sample. Researchers can use this method with longer run times to measure the distribution of folate forms in RBCs. Conclusion: This updated method allows efficient analysis of folate forms in human plasma and tissues without the loss of sensitivity or precision.

14. Simultaneous Determination of Plasma Prednisolone, Prednisone, and Cortisol Levels by High-Performance Liquid Chromatography.
Majid O, Akhlaghi F, Lee T, Holt DW, Trull A.

Abstract
Recipients of organ transplants remain particularly dependent on prednisolone as part of their maintenance immunosuppression. Despite this, the pharmacokinetics of prednisolone have never been fully characterized in these patients, and consequently dosing remains empirical. Accurate monitoring of prednisolone, its primary metabolite prednisone, and endogenous cortisol suppression in such patients may provide a means of improving the clinical outcome by adjusting for variability in prednisolone pharmacokinetics and pharmacodynamics. Measurement of endogenous cortisol may provide an independent marker of prednisolone pharmacodynamics. A simple isocratic reverse-phase high-performance liquid chromatography procedure, using betamethasone as an internal standard, was developed to quantify plasma prednisolone, prednisone, and cortisol simultaneously. The steroids were extracted from 0.5 mL plasma with 3 mL (1:1 v/v) ethyl acetate/tert-methyl butyl ether and 0.1 mL phosphoric acid, washed in 0.1 mol/L NaOH before a final drying step and reconstitution in mobile phase for injection. Separation was achieved using a Supelcosil LC-18-DB, 150 × 4.6-mm, 5-μm particle size, reverse-phase column attached to a Newguard 15 × 3-mm, RP8 guard column maintained at 25°C, with
ultraviolet detector set at 254 nm. The mobile phase consisted of 16% isopropanol in water containing 0.1% trifluoroacetic acid, set at a flow rate of 1.2 mL/min. The assay was linear up to 1,002 _g/L for prednisolone, 982 _g/L for prednisone, and 545 _g/L for cortisol. Mean intra-assay and interassay imprecision levels were 6.0% and 7.2%, respectively, for prednisolone, 5.8% and 7.2% for prednisone, and 5.6% and 7.9% for cortisol. Intra-assay inaccuracy was <7% of nominal values for prednisolone, prednisone, and cortisol. The lower limit of quantification was 7 _g/L for prednisolone and prednisone and 10 _g/L for cortisol. Corticosteroid recoveries were 73%, 74%, and 90% for prednisolone, prednisone, and cortisol, respectively. The authors describe a robust, inexpensive, and simple method suitable for therapeutic drug monitoring or pharmacokinetic studies of prednisolone; it may also be used to measure the suppression of endogenous cortisol production.

   Manish Kumar T, Srikanth G, Rao V, Rao KS.

Abstract
A simple, rapid, selective and sensitive HPLC method was developed and validated for the determination of levofloxacin from human plasma. The drug was extracted with ethyl acetate, levofloxacin was measured in plasma using a validated HPLC method with UV detector at 235nm chromatographic peaks were separated on 5µm intensil, c18 column (4.6x250mm) using 80:20v/v phosphate buffer pH2.5, Acetonitrile as mobile phase at a flow rate of 1ml/min. The chromatograms showed good resolution and no interference from plasma. The retention time of levofloxacin and internal standard were approximately 5.9±0.05min and 10.1±0.03min respectively. The mean recovery from human plasma was found to be above 85%. The method was linear over the concentration range of 0.1 to 10µg/ml with coefficient of correlation (r2) 0.9994. Both intraday and interday accuracy and precision data showed good reproducibility. This method was successfully applied to pharmacokinetics studies.

Meyyanathan SN, Muralidharan S, Rajan S, Gopal K and Suresh BA. 

Abstract 

A simple, rapid and selective method was developed for the estimation of amlodipine from human plasma. The method involves a simple protein precipitation techniques using nifedipine as internal standard. Chromatographic separation was carried out on a reverse phase C18 column using mixture of 50 mM potassium di hydrogen ortho phosphate (pH 7.5) and acetonitrile (60:40, v/v) at a flow rate of 1.0 mL/min with UV detection at 239 nm. The retention time of amlodipine and internal standard were 4.12 and 8.31 min, respectively. The method was validated and found to be linear in the range of 0.5-50.0 ng/mL. An open, randomized, two-treatment, two period, single dose crossover, bioequivalence study in 24 fasting, healthy, male, volunteers was conducted. After dosing, serial blood samples were collected for the period of 168.0 h. Various pharmacokinetic parameters including AUC0–t, AUC0–∞, Cmax, Tmax, T1/2, and elimination rate constant (Kel) were determined from plasma concentration of both formulations of test (Amlodipine 5 mg tablets) and reference (Amlodipine 5 mg tablets). Log transformed values were compared by analysis of variance (ANOVA) followed by classical 90% confidence interval for Cmax, AUC0–t and AUC0–∞ and was found to be within the range. These results indicated that the analytical method was linear, precise and accurate. Test and reference formulation were found to be bioequivalent.

17. Pharmacokinetics of celecoxib in the presence and absence of interferon induced acute inflammation in the rat: application of a novel HPLC assay. 

Micheal SG, Saeed S, Fakhreddin J. 

Abstract 

Celecoxib(CEL) is a relatively new cyclooxygenase-2 specific inhibitor nonsteroidal anti-inflammatory drug with low incidents of the toxic side effects. We developed and validated an HPLC assay for CEL and delineated pharmacokinetics of the drug in the rat in the presence and absence of inflammation. Methods. Rat plasma (0.1 mL plasma) was spiked with CEL and ibuprofen as internal standard. The solution was
acidified and constituents were extracted with isoctane-isopropanol(95:5). The organic solvent was separated, evaporated and the residue was dissolved in the HPLC mobile phase [acetonitrile-water-acetic acid-triethylamine(47:53:0.1:0.03)]. The HPLC system consisted of an auto-injector, an isocratic pump, a 10cm c18 column packed with 5µm of reversed phase particles, a UV detector set at 254nm and an integrator. Control adult male Spraque-Dawley rats were dosed with CEL. Serial blood samples were collected via a inserted catheter at the right jugular vein, and plasma samples were analysed for CEL. Results. The assay yielded linear response within the examined ranges of 20-1000ng/mL and 1-100 ng/mL(r2>0.99) with an extraction efficiency of >70%, intra- and inter-day variabiility of <10% and accuracy of >90%. In control rats, CEL had an oral bioavailability of 0.59 due mainly to presystemic hepatic metabolism. A multicompartmental disposition kinetics either an average terminal t1/2 og 2.8±0.7h, and volume of distribution of 2.3±0.6L/kg were found. Acute inflammation had no significant effect on the pharmacokinetics of CEL, although a trend towards increased plasma concentration was noticed. Conclusions. The validated assay has sufficient accuracy and precision for pharmacokinetic studies of CEL in the rat. The lack of change in CEL in the pharmacokinetics after acute inflammation may be due to 1) insensitivity of its metabolic system to the acknowledged inhibitory effect of inflammation, and/ or 2) the relatively low pre-systemic metabolism of the drug.


Abstract
A high-performance liquid chromatographic method using a linear elution gradient has been developed for the analysis of sulindac, sulindac sulfone, and sulindac sulfide in plasma, urine, bile, and gastric fluid. The methodology uses reverse-phase, radial compression chromatography with gradient elution, and UV detection. Sulindac and its metabolites in plasma can be quantitated at 0.25 microgram/mL with a mean CV of 6.0 +/- 2.9%; urine, bile, and gastric fluid (0.5 microgram/mL) yield a mean CV of 5.5 +/- 1.9%.
19. Detection of Cotinine in Blood Plasma by HPLC MS/MS.

Oneil Bhalala.

Abstract

Tobacco smoking is a major killer in the United States and is attributed to approximately 434,000 deaths per year. Primary and secondary exposure to tobacco and tobacco smoke can be monitored by measuring cotinine levels in blood, urine, as well as other matrices. This article describes a HPLC MS/MS assay to detect low concentration levels of cotinine in blood plasma. The assay was developed at Children’s Hospital, Boston, and thus it was specifically designed for use with young children. This assay allows for high throughput and turnaround because it does not use a column-based purification process; it is also fairly inexpensive, using common laboratory reagents. Upon completion of the study, the concentration ranges were found to be accurate from 0.1 to 10.0 ng/mL. The limit of quantitation was calculated to be 0.2 ng/mL (CV% < 20%, accuracy range ± 20%). The HPLC MS/MS assay is now ready for comparison tests with the ELISA test using patient plasma samples.


Abstract

The study presents the development and validation of a simple HPLC method for the simultaneous determination of metformin (MTF) and gliclazide (GCZ) in the presence of glibenclamide, in human plasma, for the clinical monitoring of MTF and GCZ after oral administration or for bioequivalence studies. Ion-pair separation followed by UV detection performed on deproteinised plasma samples was chosen for the determination of metformin and gliclazide. The internal standard was glybenclamide. The HPLC method used a Zorbax Eclipse XDB-C18 150x4.6 mm i.d. (5μm) column and analytical guard column 12.5x4.6 mm (5μm), with a gradient elution (1 mL/min) at 40°C column temperature. The mobile phase was acetonitrile: methanol (1:1v/v) and sodium dodecylsulphate 5mM, pH=3.5 with H3PO4 85% and gradient elution. The eluent was monitored at 236 nm. The calibration curve was linear within the
range of 0.05-5.00 μg/mL ($r^2=0.99$, $n=6$). The lowest limit of quantification (LLOQ) was 50 ng/mL for metformin and 49 ng/mL for gliclazide. The proposed method was validated and proved to be adequate for metformin and gliclazide clinical monitoring, bioavailability and bioequivalence studies.


Ruiz-Garcia A, Bermejo M, Moss A, Casabo VG.

Abstract
The aim of this current review is to summarize the present status of pharmacokinetics in Drug Discovery. The review is structured into four sections. The first section is a general overview of what we understand by pharmacokinetics and the different LADMET aspects: Liberation, Absorption, Distribution, Metabolism, Excretion, and Toxicity. The second section highlights the different computational or in silico approaches to estimate/predict one or several aspects of the pharmacokinetic profile of a discovery lead compound. The third section discusses the most commonly used in vitro methodologies. The fourth and last section examines the various approaches employed towards the pharmacokinetic assessment of discovery molecules; including all the LADME processes, discussing the different mathematical methodologies available to establish the PK profile of a test compound; what the main differences are and what should be the criteria for using one or another mathematical approach. The major conclusion of this review is that the use of the appropriate preclinical assays has a key role in the long-term viability of a pharmaceutical company since applying the right tools early in discovery will play a key role in determining the company's ability to discover novel safe and effective therapeutics to patients as quickly as possible.

22. Simultaneous HPLC determination Of isoniazid and acetylisoniazid in plasma.

Segovia RM, Flores P, Torres JD, Ramirez XH. Perez V, Romano S.

Abstract
A rapid and simple high-performance liquid chromatographic method has been developed for simultaneous determination of isoniazid (INH) and acetylisoniazid (AcINH) in microsamples of plasma. Plasma samples were deproteinated by addition of trichloroacetic acid, and the drug and its metabolite were then separated by
reversed-phase HPLC on an octade-cylsilane-bonded silica column. The mobile phase was a gradient prepared from an aqueous solution of 1-hexanesulfonic acid sodium salt (pH 3) and acetonitrile. The effluent was monitored by UV detection at 290 nm. Calibration plots were linear in the range 0.5 to 15.0 μg mL\(^{-1}\) and the limit of quantification was 0.5 μg mL\(^{-1}\) for both drugs. The lower limits of detection for INH and AcINH were 0.24 and 0.12 μg mL\(^{-1}\), respectively. Results from analysis of quality-control samples at concentrations of 0.8, 5, and 13 μg mL\(^{-1}\) were indicative of good repeatability and precision. Recovery from plasma was 64% for INH and 55% for AcINH. INH was more stable than AcINH in plasma at –80°C.

23. Determination of lomefloxacin in biological fluids by high-performance liquid chromatography and a microbiological method.

Shibl AM, Tawfik AF, el-Houfy S, al-Shammary FJ.

**Abstract**

A high-performance liquid chromatographic method (HPLC) was developed for the determination of lomefloxacin in plasma and urine and was compared to a microbiological assay. Lomefloxacin and norfloxacin (internal standard) were extracted from plasma and urine samples using chloroform. Measurements were carried out with a fluorescence detector using an excitation wavelength of 280 nm and an emission wavelength of 430 nm with a mercury lamp. Quantification was achieved by the measurement of the peak-height ratio and the analytical recovery of the drug from plasma and urine was found to be (mean +/- SD) 99.3 +/- 3.74% and 95.7% +/- 3.82%, respectively. In the microbiological assay, E. coli ATCC 1346 was the test organism using an agar diffusion technique. The coefficients of variation for within-day analysis for both the HPLC method and microbiological assay from plasma samples were less than 7%. The minimum detectable concentration for both the HPLC and the microbiological method was 50 ng/ml and 100 ng/ml, respectively. Both methods were used to determine the lomefloxacin level in plasma following intravenous administration to mice. Excellent agreement was obtained between the results of the two methods. The HPLC method offers significant advantages in accuracy, precision, speed of analysis and turnover-time.
24. Validation of levofloxacin HPLC assay in plasma and dialysate for pharmacokinetic studies.

Siewart S.

Abstract
An HPLC method with fluorescence detection suitable for routine determination of levofloxacin in plasma and dialysate has been validated. Sample preparation was assured by one-step protein precipitation for plasma or direct injection of the dialysate solution, respectively. Separation occurred on an YMC Pro C18 RP column (150 mm × 2 mm) with an acidic binary gradient mobile phase and a detection at excitation and emission wavelengths of 296 and 504 nm. The assay was linear between 0.1 and 6 Â¼g/ml for plasma and 0.1 and 5 Â¼g/ml for dialysate with intra- and inter-day precision and accuracy lower than 10%. No degradation of levofloxacin was observed under the applied conditions for both matrices. The method was successfully applied to an in vitro pharmacokinetic study and patient samples as well.

Solomon BP, Duda CT.

Abstract
Recent publications suggest that high homocysteine levels are either 1) the cause of heart disease, 2) an independent risk factor for heart disease, 3) the result of heart disease, or 4) unrelated to heart disease. In any event, for both clinical and research purposes, a reliable method is needed for the determination of homocysteine in plasma. BAS launches its Total Plasma Homocysteine Kit to fill the need for collection of definitive data in larger populations. The kit is based on liquid chromatography with electrochemical detection (LCEC) and exhibits >97% recovery, with among-day RSD of 0.9 - 1.9% and within-day RSD of 0.3 - 0.4%.

Sriwiriyajan S, Mahatthanatrakul W.

Abstract
The objective of this study was to develop a rapid and simplified, reliable high-
performance liquid chromatography (HPLC) method for quantification of cefpirome (CAS 98753-19-6) in plasma. After precipitation of the plasma containing the internal standard, hydrochlorothiazide, with 5% trichloroacetic acid (TCA), the analysis of the cefpirome level in the plasma samples was carried out using a reverse-phase C18 column with the ultraviolet detector set at a wavelength of 258 nm. The chromatographic separation was accomplished with an isocratic mobile phase consisting of acetonitrile-acetate buffer pH 5. The proposed method was specific and sensitive with a lower limit of quantitation (LLOQ) of 0.5 microg/ml. This HPLC method was validated by examining the precision and accuracy for inter- and intra-day analysis in the concentration range 0.5-64.0 microg/ml. The relative standard deviation in the inter- and intra-day validation was less than 3%. Analytical recovery was more than 84%, and cefpirome was found to be stable in human plasma during both the storage and assay procedures. A satisfactory pharmacokinetic study of cefpirome was carried out in rabbits using the devised procedure.

27. Development and validation of high performance liquid chromatographic method for the simultaneous determination of ceftriaxone and vancomycin in pharmaceutical formulations and biological samples.

Abstract
A reverse phase-liquid chromatographic method with UV detection at 280 nm is described for simultaneous determination of ceftriaxone sodium and vancomycin hydrochloride. Chromatographic separation of the two drugs was achieved on a Betasil C-1 column using a mobile phase consisting of a binary mixture of acetonitrile and triethylamine buffer adjusted to pH 3.5_0.1 with orthophosphoric acid in a ratio of 20:80. The liquid chromatographic method developed offers symmetric peak shape, good resolution, and reasonable retention time for both drugs. Linearity, accuracy, and precision were found to be acceptable over the concentration ranges 125–750 ppm for ceftriaxone and 62.5–375 ppm for vancomycin. The liquid chromatographic method was successfully applied to the quality control of formulated products, plasma, and cerebrospinal fluid samples containing ceftriaxone and vancomycin.
   Tian Y, Wang Q, Yang W, Kong D, Zhang L.

Abstract
To develop a sensitive, simple, and accurate method for the determination of shionone in rat plasma after ig administration of Asteris Radix petroleum ether extract (RAPE).

Methods The separation was achieved by HPLC on a RP18 column (150 mm x 3.9 mm, 5 μm) with a mobile phase composed of acetonitrile-0.05% phosphoric acid water (98:2) at a flow rate of 1.0 mL/min. UV Detector was set at 200 nm and friedelin was chosen as an internal standard. Results The linear range of the standard curves was (0.3443–22.0) μg/mL with the correlation coefficient of 0.9968. The intra- and inter-day precisions were all below 10% and the relative error was −3.5%–1.1%. Conclusion The developed method can be successfully applied to the pharmacokinetic study. After ig administration of RAPE, T1/2(ka) is (33.09 ± 7.32) min and T1/2(ke) is (84.95 ± 22.34) min.

29. Bioanalytical method development and validation For the simultaneous estimation of lamivudine And stavudine in human plasma by HPLC.
   Verma S, Mullick P, Bhatt S, Siddiqui N, Alam O, Bala I, Khan SA.

Abstract
Lamivudine (Fig. 1), chemically 2-f,3-f-dideoxy-3-f-thiacytidine, is a pyrimidine analogue, whereas stavudine (Fig. 1), 2-f,3-f-didehydro-3-f-dideoxythymidine, is a thymidine analogue. Both are reverse transcriptase inhibitors reported to be active against HIV-1, HIV-2 and hepatitis B virus. (α) Enantiomer of lamivudine has less cytotoxic and greater antiviral activity than its (+) enantiomer. It shows synergistic effect with other antiretroviral agents including stavudine, zidovudine and nevirapine (1, 2). Lamivudine and stavudine are the first line regimens in HIV treatment (3). Various methods have been used for quantitative determination of these two drugs individually in the human plasma, such as HPLC and HPLC with tandem MS (4–6), but for the combination no such method has been reported yet. The rationale of the present study was to develop an accurate, rapid and economical method for bioanalysis of the above combination of drugs in human plasma (7, 8).

Abstract
Tiotropium bromide, a long-acting inhaled bronchodilator analogous to ipratropium bromide, is currently undergoing development for the treatment of chronic obstructive pulmonary disease. To evaluate its systemic absorption in humans, we have developed a rapid and sensitive method for its determination in human plasma based on high-performance liquid chromatography with tandem mass spectrometric detection (HPLC/MS/MS). Reversed-phase chromatography of tiotropium and the internal standard clenbuterol was carried out using acetonitrile/10 mM ammonium acetate (1% formic acid) 40:60 as mobile phase in a run time of 3.0 min. The sample preparation involved deproteination with acetonitrile, extraction into dichloromethane and back-extraction into hydrochloric acid. The assay was linear over the concentration range 0.500-50.0 pg/mL with intra- and inter-day precision (as relative standard deviation) both ≤7.34%. The method was successfully applied to a pharmacokinetic study of systemic absorption in healthy male volunteers given a single 18 microg inhaled dose.

   Wanjari MM, There AW, Tajne MR, Chopde CT and Umathe SN.

Abstract
A simple reverse phase HPLC method has been developed for determining the concentration of metformin in rat plasma. The method employs C18 column (300mm X 2.4mm), ammonium acetate(0.15M) and acetonitrile(90:10; pH-5.5; 1.0mL/min) as mobile phase and ultraviolet detection at 236nm. Acetonitrile was used to simultaneously deproteinize rat plasma and extract metformin. The assay was linear in the concentration range of 0.33µg- 16.6µg/ml with co-efficient of correlation 0.994. The retention time was 4.7min. The method was found to be precise (%CV<15%).
accurate and suitable for pharmacokinetic study of orally administered metformin in rats.

Zhao B, Than S, Lu J, Lai MH, Lee KH, Moochhala SM.

Abstract
To develop a high-performance liquid chromatography (HPLC) method with photodiode-array ultraviolet detection for the simultaneous determination of vitamin C, vitamin E and β-carotene. METHODS: Following liquid-phase extraction from the human plasma samples, these three vitamins were successfully separated on the LiChrospher 100 RP-18 column (125 × 4 mm I.D.; particle size, 5 mm) at a flow-rate of 1.2 ml/min, with a mobile phase of methanol-acetonitrile-tetrahydrofuran (75: 20: 5, v/v/v). Results: The limits of quantitation were 100, 0.25 and 0.25 mg/ml for vitamin C, vitamin E and β-carotene respectively. The method is linear over the studied range of 0.25 to 5 g/ml for vitamin E and β-carotene and 100 to 5000 mg/ml for vitamin C. The extraction recoveries were greater than 83% for these three vitamins. The within day and between-day precision of the analysis did not exceed 15.3 and 16.2%, respectively. CONCLUSION: A suitable method to determine the concentration of vitamin C, vitamin E and β-carotene following oral administration of antioxidant supplement capsules to a healthy Chinese volunteer.
2.4 REFERENCES


Satyendra D et al reported Syntheses, characterization and in-vitro anti-inflammatory activity of some novel Thiophenes.”


