

Acknowledgement

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ABSTRACT

The aim of this study was to optimize, develop and validation chromatographic and spectroscopic methods for the analysis of atorvastatin calcium, losartan potassium and their degradation products using an experimental design.

The methods developed and optimized applying factorial design approach ; 2^3 which the base 2 denote to levels and exponent 3 denote to the factors. High performance chromatograph was used and the separation achieved by column C18 the detection wavelength was 246nm. A linear response was observed in the range of 6.4 $\mu\text{g/ml}$ -9.6 $\mu\text{g/ml}$ with correlation coefficient of 0.999 for method No1 and 0.9998 for method No2 of analysis of atorvastatin calcium.

The methods were validated for precision, accuracy, robustness and recovery. The methods were found to be reproducible from statistical data generated.

High performance liquid chromatograph (HPLC) was found to be stability-indicating method for the quantitative analysis of atorvastatin calcium in presence of its thermal, acid and photodecomposition hydrolysis products.

The studies of photolysis and thermal degradation of atorvastatin calcium in acid medium showed first order reaction kinetic and zero order reaction kinetic in alkaline medium.

First derivative technique ID₂₃₆ was found to be suitable for estimation of atorvastatin calcium in bulk and pharmaceutical dosage form. The percentage recovery value 99.82% indicates the accuracy of the method and absence of interference of the excipients present in the formulation.

The investigating of pharmaceutical excipients were found to decrease the thermal stability of atorvastatin calcium with decreasing the pH of excipients. The stability of atorvastatin calcium was in the following order: Citric acid, magnesium stearate, microcrystalline cellulose, lactose, tween 80, calcium carbonate, disodium phosphate and magnesium hydroxide. Atorvastatin calcium was found to be more stable at accelerating stability study for six months, but when exposed to direct sunlight showed fast degradation in liquid state.

The methods were validated for the determination of losartan potassium using the same column of determination atorvastatin and same factorial design, the methods were satisfactory with correlation coefficient in the range 6.4 to 9.6 µg/ml at wavelength 225 and 250nm respectively.

First derivative spectroscopy a signal at ID₂₃₄ nm was found to be adequate for quantification of losartan potassium.

Pharmaceutical excipients for losartan potassium formulation in tablets were investigated as the function of pH of excipient; when the pH decreased the degradation of losartan decreased also in the following order: povidone K 30, magnesium stearate, microcrystalline cellulose, lactose, maize starch and talc powder.

Losartan potassium was found to be stable drug toward stress testing, except when conducted at accelerating stability for six months it showed significant degradation.

الخلاصة

هدفت الدراسة علي تحسين وتطوير طرق تحليل عقاري الأتورفاستين كالسيوم والوسارتان بوتاسيوم والتحقق من مصداقيتها في وجود نواتج تحللها ، وكذلك تحديد العوامل التي تؤثر علي طريقة الفصل وذلك بإستخدام التجارب العامليه.

تم تطبيق التصميم العاملي 2^3 لثلاث عوامل ذات مستويين حيث تمثل القاعدة 2 المستويات و 3 تمثل الأس لثلاث عوامل مختلفة . وذلك علي جهاز الكروماتوغرافيا السائلة ذات الاداء العالي وتم الفصل باستخدام عمود فصل C18 عند طول موجي 246 نانوميتر ولقد لوحظ وجود إستجابة خطية في حدود 6.4 – 9.6 ملجرام /مل مع معامل ارتباط 0.999 بالنسبة للطريقة الاولي و 0.9998 بالنسبة للطريقة الثانية لتقدير عقار الأتورفاستين كالسيوم .

تم التحقق من مصداقيه ودقة ومثانة الطرق المطورة ومدي مناسبتها للتحليل الروتيني وكذلك تحليل نواتج التحلل . من البيانات الاحصائية ثبت أن طريقة التحليل بواسطة الكروماتوغرافيا السائلة ذات الاداء العالي دالة علي الثبات ويمكن استخدامها في التحليل الكمي لعقار الاتورفاستين في وجود نواتج تحلله الحراري وكذلك تحلله الحامضي والضوئي .

كل التفاعلات الضوئية والحرارية التي تمت دراستها علي الأتورفاستين في الوسط الحامض

أظهرت تفاعل من النظام الاول وكذلك تفاعل من الدرجة صفرية في الوسط القاعدي

تعتبر تقنية الاشتقاق الطيفي طريقة مناسبة لتقدير عقار لأتورفاستين كالسيوم في صورته الخام وكذلك في شكله الصيدلاني حيث وجد أن نسبة الاسترداد 99.8% تدل علي دقه وعدم تداخل السواغ الصيدلانية.

ثبت أن كل السواغ الصيدلانية والتي تمت دراستها —————ها تزيد من التحلل الحراري لعقار الأتورفاستين مع انخفاض الاس الهيدروجيني وكان عدم الاستقرار علي النحو التالي :

حامض الستريك ، سترات الماغنسيوم ، السيلولوز توين 80 ، كربونات الكالسيوم ، فوسفات الصوديوم ، وهيدروكسيد الماغنسيوم .

وهذا يدل علي أن الاس الهيدروجيني في القيم الدنيا يزيد من التفكك الحراري لعقار الأتورفاستاتين كالسيوم ، عقار الأتورفاستاتين أظهر استقرار جيد عند دراسة الثبات المتسارع لمدة ستة أشهر مع الأخذ في الاعتبار أن ضوء الشمس المباشر أثر عليه بصورة كبيرة في حالته السائلة .

تم تطوير طرق تحليل عقار اللورستان بوتاسيوم بنفس عمود فصل الأتورفاستاتين السابق وبنفس التصميم العملي حيث وجد أن الطرق تعتبر مرضية وكافية لتقدير عقار اللوسارتان بوتاسيوم حيث اعطت كل الطرق علاقة خطية في المدى 6.4 – 9.6 مليغرام/مل عند طول موجي 250 نانوميتر و 225 نانوميتر علي التوالي .

وجد أن تقنية الاشتقاق الطيفي عند طول موجي ID234 كمشتقة اولي تعتبر طريقة مناسبة لتقدير عقار اللوسارتان بوتاسيوم في شكله الصيدلاني .

ثبت أن السواغ الصيدلانيه التي تمت دراستها في هذا البحث بالنسبه لعقار اللوسارتان بوتاسيوم تساعد علي الثبات بزيادة الاس الهيدروجيني علي النحو التالي :-

بوفيدون ك 30 ، ماغنسيوم ستياريت ، السيلولوز ، اللاكتوز ، والنشأ، وبدرة التلك .

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Layout of Thesis

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List of Abbreviations

ICH	International Conference of Harmonization
FDA	Food and Drug Administration
IR	Infrared
FTIR	Fourier transform infrared spectroscopy
HPLC	High Performance Liquid Chromatography
B.P	British Pharmacopeia
USP	United states Pharmacopeia
API	Active pharmaceutical ingredient
UV	Ultraviolet
VIS	Visible light
ODS	Octadecyl silane
RI	Refractive index
FID	Flame ionization detector
THF	Tetrahydrofuran
LOD	Limit of detection
LOQ	Limit of quantitation
NA	Not applicable
DP	Drug product
CDER	Central of drug evaluation and research
RP	Reverse phase
HPTLC	High performance thin layer chromatography

SEM Standard error of the mean

List of Publications

Rudwan, E.H and Hussein, A.B.E.M and .Saeed, A. E.M. **(2015)**. Development and validation of Stability-indicating High Performance Liquid chromatography Method for determination of atorvastatin Calcium in the presence of its degradation products. *Der pharma Sinica*, 6 (11):19-27.

Rudwan, E.H.; Hussein, A.B.E.M. **(2015)** .Development and Validation of stability-indicating method for determination of atorvastatin calcium in pharmaceutical dosage forms. *Journal of chemical and pharmaceutical Research*, 7(11):813-822.

Rudwan, E.H. **(2015)**. Development and validation of an UV derivative spectrophotometric determination of atorvastatin calcium in bulk and pharmaceutical formulation. *Journal of chemical and pharmaceutical Research*, 7 (11):823-828.

Hussein, A.B.E.M. and Rudwan, E.H. **(2015)**.Development and validation of an UV derivative spectrophotometric determination of losartan potassium in tablet. *Der pharma Chemica*, 7(12):175-180.

Rudwan, E.H.; Hussein, A.B.E.M and.Saeed, A. E.M. **(2016)**. Development and validation of stability indicating method for analysis of losartan potassium in bulk drug and dosage form formulation. *International Journal of pharmaceutical science and Research*; 7(4): 1413-1421.

Mohammed E.W.B Amna, and Rudwan, H.E. **(2016)**. RP-HPLC method development and Validation of Stability –indicating Method For estimation of losartan potassium under stress condition and tablet dosage form. *International Journal of pharmaceutical science and Research*. 7(6): 2343-2351.