بسم الله الرحمن الرحيم

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شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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قسم

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Novel HPLC methods for the assessment of Febuxostat and Allopurinol

A Thesis
Submitted to Chemistry Department – Faculty of Science – Ain Shams University in Partial Fulfillment for Requirements of the Master Degree of Science (M.Sc) in Chemistry

By

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Approval sheet

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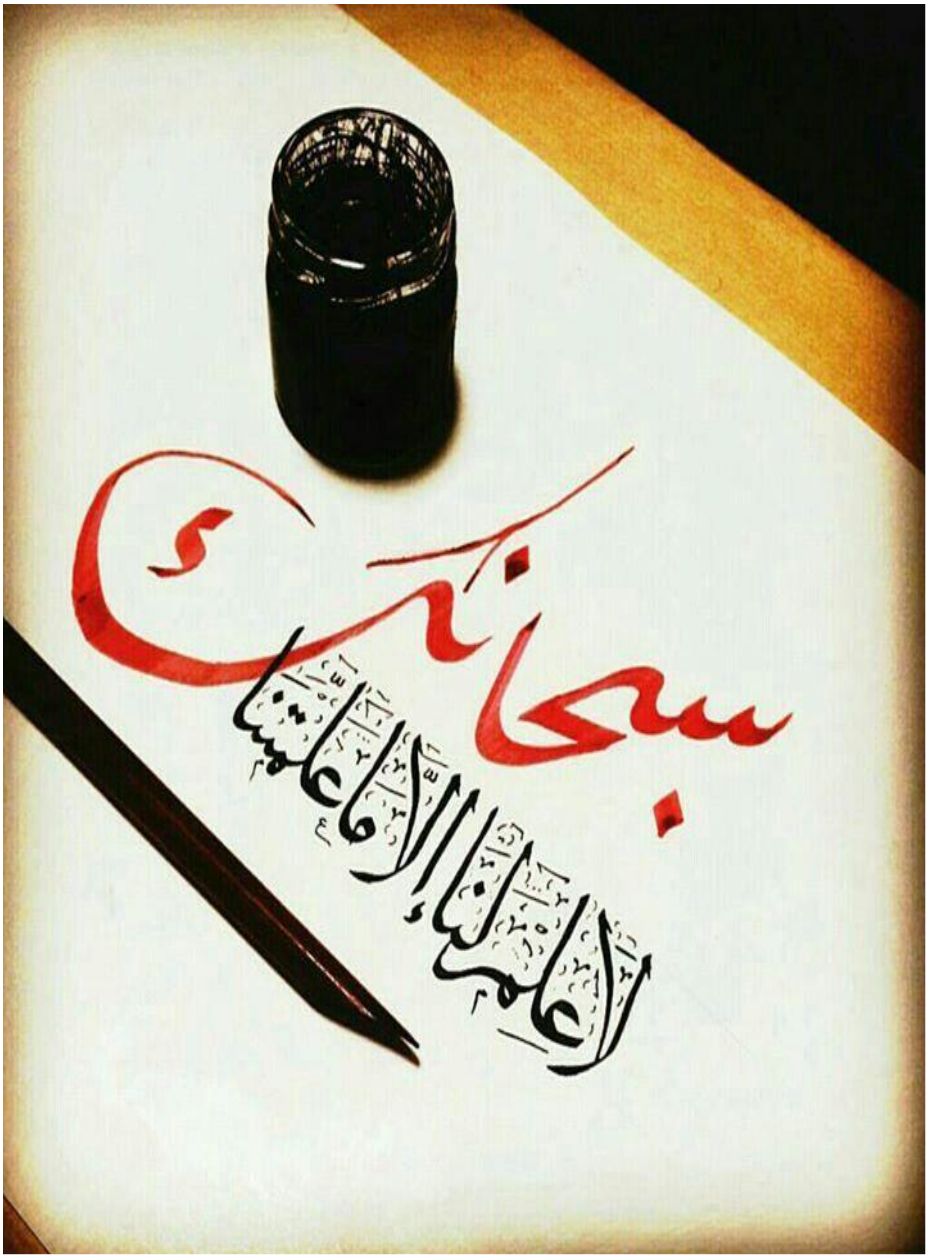
سبحانك
لا علم لنا إلا ما علمتنا

Dedicated with love

to my loving parents

to my lovely wife

to my lovely son

to all my family

to my best friends

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Ahmed Mohamed Ahmed Farag

Summary

Summary

Three specific, highly sensitive and rapid RP-HPLC methods have been developed for the assessment of Febuxostat and Allopurinol in their pure and bulk pharmaceutical dosage forms based on UV or fluorescence detection.

The first method describes the chromatographic separation and assessment of Febuxostat using an Agilent 1260 infinity series HPLC system equipped with an Agilent Eclipse plus C_{18} column (250 X 4.6 mm, 5µm particle size) at 45 ^{o} C and a UV detection at a wavelength of 318 nm. The mobile phase was 80 : 20 v/v% of acetonitrile : 5 mmol/L acetic/acetate buffer of pH 3.30 at a 0.75 mL/min flow rate, in the isocratic flow mode. The retention time was 5.1 ± 0.1 min. A 20 µl injection volume of three replicates was applied. A linear calibration graph was obtained for peak area versus Febuxostat concentration for 0.0 – 90.0 µg/mL Febuxostat with a regression equation of A = 127.81X + 12.55 and a correlation coefficient of 0.9999. The average recovery was 99.09%. The limit of detection and lower limit of Quantitation were 1.0 and 3.0 µg/mL, respectively. The method was conveniently applied to the assessment of Febuxostat in elven commercially available pharmaceutical formulations.

The second method describes the chromatographic separation and assessment of Allopurinol using an Agilent 1260 infinity series HPLC system equipped with Prontosil cyano column C_{18} column (150 X 4.6 mm, 5µm particle size) at 35 ^{o} C and a UV detection at a wavelength of 250 nm. The mobile phase was 40 : 60 v/v% of methanol : 25 mmol/L KH_2PO_4 buffer of pH 4.60 at a 0.50 mL/min flow rate, in the isocratic flow mode. The retention time was 4.45 ± 0.10 min. A 20 µl injection volume of three replicates was applied. A linear calibration graph was obtained for peak area versus Allopurinol concentration for 5.0 – 90.0 µg/mL Allopurinol with a regression equation of A = 140.37X + 51.79 and a correlation coefficient of 0.9996. The average recover was 99.40%. The limit of detection and lower limit of Quantitation were 1.6 and 4.8 µg/mL, respectively. The method was conveniently applied to the assessment of allopurinol in eight commercially available pharmaceutical formulations.

The third method describes the chromatographic separation and assessment of Allopurinol using an Agilent 1260 infinity series HPLC system equipped with X Select Waters HSS - C_{18} column (150 X 4.6 mm, 5µm particle size) at 25 ^{o} C and a spectrofluorimetric detection. Allopurinol was derivatized by its reaction with the fluorogenic reagent NBD-Cl (4-chloro-7-nitrobenzo-2-oxa-1,3-diazole) and measured at λ_{ex} Excitation wavelength 468 nm and λ_{em} Emission wavelength of 535 nm. The

mobile phase was 30 : 70 v/v% of methanol : 0.10% trifluoroacetic acid at a 1.0 mL/min flow rate, in the isocratic flow mode. The retention time was 7.32 ± 0.10 min. A 20 µl injection volume of three replicates was applied. A linear calibration graph was obtained for peak area versus Allopurinol concentration for 0.06 – 3.6 µg/mL Allopurinol with a regression equation of A = 56.36X + 101.9 and a correlation coefficient of 0.9997. The average recover was 101.5%. The limit of detection and lower limit of Quantitation were 0.08 and 0.25 µg/mL, respectively. The nature of the derivatization product was inferred. The method was conveniently applied to the assessment of allopurinol in eight commercially available pharmaceutical formulations.

The developed methods were compared with the existing standard pharmacopieal methods and there were no significant differences between the means and variances of the data of the standard and developed methods confirming the validity of the proposed methods.

Introduction and Literature Review

Literature Review of Allopurinol

HPLC Methods

A reversed phase high performance liquid chromatographic method was described by Kramer and Feldman (1979) for the determination of Allopurinol and oxipurinol in blood plasma. As little as 0.1 mL plasma sample was deproteinated with trichloroacetic acid and acetaminophen was used as an internal standard. The separation was achieved on Spherisorb ODS (5 μm) column using a phosphate buffer mobile phase, with a UV detection at 254 nm. A linear calibration graph was obtained for 0 - 20 μg/mL with a limit of detection of 0.1/μg/mL. Strong interference was observed from 6-Mercaptopurine that exhibited the same retention time as allopurinol.

A RP-HPLC method was described by Wung and Howell (1980) for the assesment of 5-fluorouracil, uridine, hypoxanthine, xanthine, uric acid, Allopurinol, and oxipurinol in biological fluids of plasma and serum. A 50 mmol/L phosphate buffer of pH 4.60 was used as an eluent on a μBondapak C_{18} column.

A RP-HPLC method was described By McBurney and Gibson (1980) for the quantification of pyrimidine, purine and pyrazolopyrimidine nucleosides and bases. Gradient elution was achieved on C_{18} μ-Bondapak column with dihydrogen Phospate / methanol as an eluents and a 254 nm UV detection. This method allowed the assessment of purine and pyrimidine metabolites in