Novel and high sensitive quantitative analysis of Phenacetin using Liquid Chromatography Triple Quadrupole Mass Spectrometry

Graduate Students, Science and Engineering

Fathy A. Atia*, A. A. Ahmadi; M. Al-Safran

Central Laboratories Unit, Office of Research, Qatar University, State of Qatar – Doha. P.O. Box: 2713, Email: fathy.ghaly@qu.edu.qa

Abstract

A lowest detection limit with straight linearity were obtained by developing a method to analyze Phenacetin (Phe) in both aqueous and organic extraction by using Liquid chromatography Triple Quadrupole mass with electrospray ionization (LCMSMS/ESI). The validation of the developed method was carried out according to ICH Harmonized Tripartite guideline. Validation criteria obtained were; the method detection limit MDL is 0.057 ng/ml, method quantification limit MQL is 0.1 ng/L while the calibration curve linear from 0.1 to 500 ng/L with correlation coefficient R2 is 0.9994, Accuracy and precision from 97 to 108% at inter and intraday for six replicates of three concentrations with RSD 2.1%. Separation occurred using Zorbex C18 4 um, 150 x 2.1 mm column, using acetonitrile : 0.1% Formic acid 60:40% (v:v) at flow rate 1ml/min. the detector was triple quad mass spectrometry at multi-reaction mode MRM to detect parent mass 180.1 at frag 97 to transition fragments 110 and 138 at collision voltages 16 and 12 respectively.

Introduction

Phenacetin or p-ethoxyacetanilide had been a commonly used as analgesic antipyretics drug (Hinson, 1983). It is highly lipid-soluble with limited aqueous solubility, and its oral absorption is highly dependent on formulation factors such as particle size (PRESCOTT L. S., 1970). The drug is extensively metabolized and less than 0.5% of a dose is recovered unchanged in the urine (PRESCOTT L. , 1980).

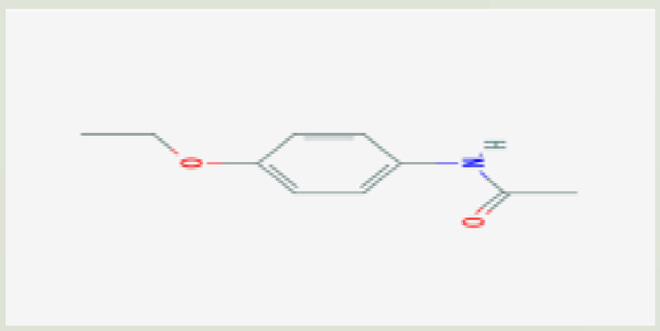
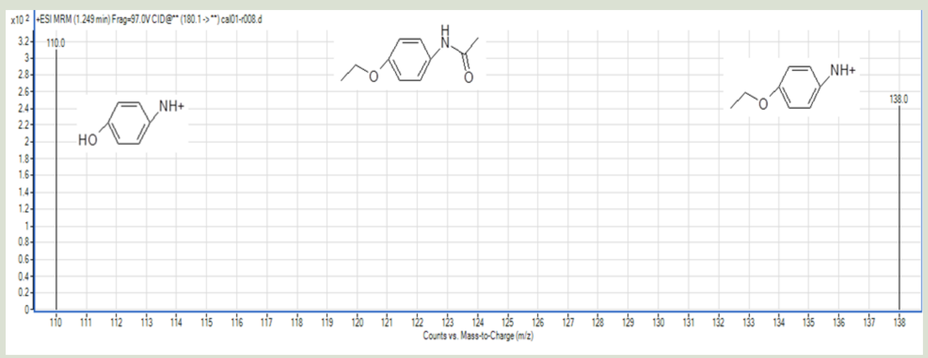


Figure 1: Phenacetin structure

Methodology

Separation by Liquid chromatography

Agilent 1290 infinity UHPLC system, equipped with a binary pump, thermostated column compartment, micro-vacuum degasser and auto-sampler was used. Analytical separation column was Nova pack® C18 (4µm, 3.9 x 150 mm). Isocratic elution consists of 40% acetonitrile as solvent A and 60% of 0.1% formic acid as solvent B at 1ml/min flow rate. Injection volume was constant for all sample injection at 5uL. Mass Hunter acquisition and quantification software were used for data analysis.

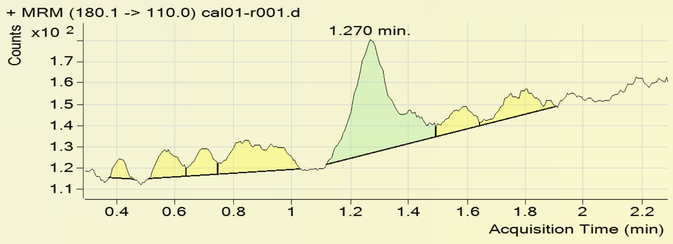


Mass Spectrometry

Agilent 6460 Triple Quad with positive electrospray ionization (+ESI) was used. Nitrogen from Peak scientific generator used as cone and desolvation gas at flow rate 7L/min and as nebulizer at 45 psi. Gas temperature was 320oC while sheath gas temperature was 350 oC. Sheath gas flow was 11 L/min. capillary voltages were 3500 for positive and 4500 for negative at 63nA. Multi-reaction monitoring (MRM) mode sets for transition 180.1 110 at collision energy (C.E) 16 and 180.1 138 at C.E 12 while fragment or was stable at 97.

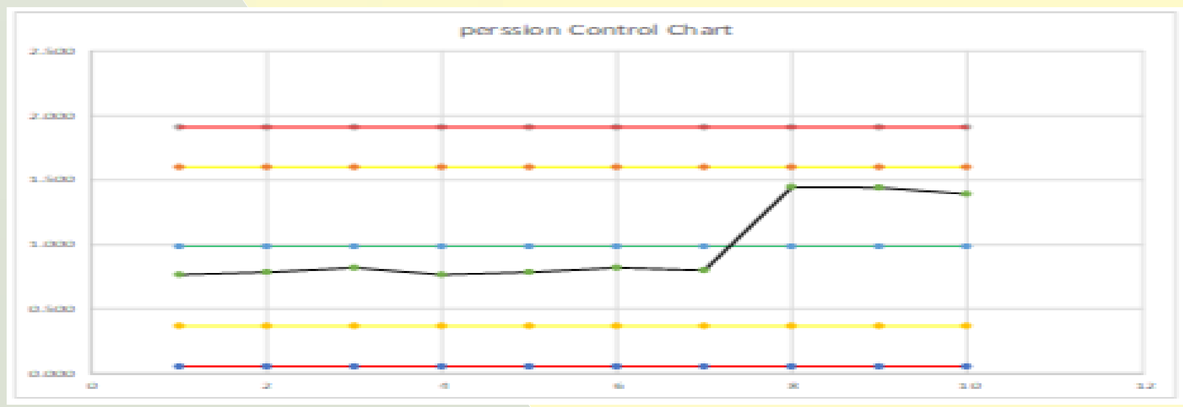
Method Validation

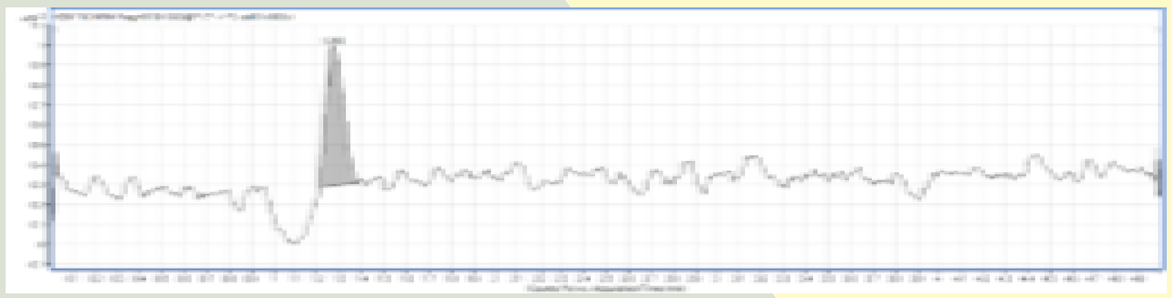
The developed method was validated regarding to limits of detection and quantification (LOD & LOQ), linearity, specificity, accuracy, precision, recovery percentage and robustness as the ICH guideline.



Conc	Accuracy %	RSD%
at 0.1	166.97	1.2
at 1	98.24	3
at 10	117.73	24

LOD	0.0893 ng/ml
LOQ	0.1907 ng/ml





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