Method Development for Simultaneous Estimation of Acetaminophen and Its Related Impurities by Liquid Chromatography-mass Spectrometry

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Short Report

Keywords: Liquid chromatography Mass spectrometry, Acetaminophen, related substance, Method development, Identification.

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METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF ACETAMINOPHEN AND ITS RELATED IMPURITIES BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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ABSTRACT

Development and Analysis of drugs involve the most stimulating work in the medicinal field in that way, in that way, there emergence of new drug in day to day . Acetaminophen is a common Over the counter drug used by worldwide as the use of this drug increased globally requirement for detecting its impurities is mandatory and its determination of related substance were evaluated by using Liquid Chromatography Mass spectrometry, this method has emerged as a capable and multipurpose tool for analysing various types of drug product and its impurities. It has the capability of detecting small molecule to high molecule with high sensitivity. The optimized method was developed for estimation of related substance of acetaminophen using a gradient program mobile phase of formic acid and acetonitrile with 0.6 ml/min flow at 210 and 254 nm wavelength with optimized mass condition. In this paper, briefly discussed about method development for determination of related substance of acetaminophen along with its active pharmaceutical ingredient in a finished marketed product.

KEYWORD

Liquid chromatography Mass spectrometry , Acetaminophen , related substance, Method development ,Identification.

1. INTRODUCTION [1,2]

Pharmaceutical analysis is one of most interesting branches in the medicinal field because it involves the qualitative and quantitative analysis of compound or drug product using various analytical techniques. The analysis of drugs reveals about the strength, purity and its nature There are various types of analytical techniques like chromatographic technique, spectroscopic technique, elemental analysis, thermal analysis etc., Chromatographic techniques are the techniques in which separation of compounds from a mixture using mobile phase and stationary phase, whereas Spectroscopic techniques are used to study nature of components by using Electromagnetic radiation.

Hyphenated techniques are the analytical method in which chromatographic technique coupled with spectroscopic technique Chromatographic techniques are used to separation of compound whereas spectrometric technique is used to identification of compound [for e g. LC-MS, MS-MS, GC-MS, LC-NMR,CE-MS].LCMS {Liquid Chromatography Mass Spectrometry}is one of the hyphenated techniques used to separation of compound, followed by identification. It also used for determination of molecular weight, purity etc.,

Acetaminophen or commonly known as paracetamol (structure as shown fig 1) , its most popular OTC drug used as an antipyretic, analgesic etc... acetaminophen is a quickly and completely absorbed in systemic circulation after oral administration (bioavailability 90%). As the use of paracetamol drug increased globally requirement for detecting its impurities is globally important. Impurities are the unwanted chemicals that present in drug product , that may be classified into organic impurity, inorganic impurity and residual solvents .Related substances are the organic impurities present in the drug that may arise from the raw materials, process related by-products, degradation products. Several methods had been development for determination of its related substance by

HPLC . In this article we discussed about the a simple, and a single a novel method is used for determination of impurities present in acetaminophen tablets by LCMS method.

Analytical method development is the process of selecting and optimizing analytical methods to measure the specific attributes of a drug substance or product. Analytical method development are critical tools for ensuring the quality, safety and efficacy of the drug product. The goal of the method development is to ensure that the methods are used to identity, purity and reliable. Analytical method development is the creation of a set of experimental condition to perform analytical procedures in chemical samples. Developed analytical methods can be used to identify, separate and used to learn more about the chemical components in drug components .The steps involved in the method development are [3,4,5]

- ✓ Define a objectives of method development
- ✓ Collect the literature survey
- ✓ Develop the method plan.
- ✓ Optimize the method
- ✓ Validate the method
- ✓ Sample analysis

2. EXPERIMENTAL DESIGN

2.1 CHEMICAL REAGENTS

Acetaminophen tablets 500 mg tablets of (Batch no: APCI 0720 02) purchased from market, Formic acid purchased from Honeywell having MS grade, Acetonitrile purchased from MERCK having MS grade and Methanol from MERCK having MS grade and Deionised water were purified using milli Q water used for this project.

2.2 LC-MS INSTRUMENTATION AND CONDITION

The liquid chromatographic techniques and mass spectrometry were achieved by coupling waters made HPLC with mass spectrometry (waters USA). LC having quaternary pump of automated sample injection with thermostat column temperature of STATIONARY PHASE Column – waters, C18, 100^{x}4.6mm, 5µ with a dual wavelength 210 nm and 254 nm of 0.6 ml/min flow rate with gradient mobile phase programme (shown in table 1) with injection volume of 20µl.

Mass spectrometry electron spray ionization technique with condition being achieved by proper selection of desolvation temperature 400°C, source temperature 130°C with optimum gas flow (Nitrogen) 800 L/HR., cone voltage 30V.

2.3 PREPARATION OF SOLUTION

2.3.1 Preparation of mobile phases:

Mobile phase A: Acetonitrile (100%)

Mobile phase B: 0.1% Formic acid :Transferred 1ml of formic acid in 1lit of water and mixed well

2.3.2 Preparation of diluent:

Mixed methanol and water in equal volumes (50:50)

2.3.3 Preparation of Sample Solution

Weighed 20 tablets and crushed into fine powder. Weighed 10 mg of powdered sample and transferred into 10 ml flask (concentration having 1mg/ml) and diluted with diluent and filtered and injected.

3.RESULT AND DISCUSSION

By above mentioned parameters method was developed and. Successfully determined the related substance of acetaminophen in a finished product as complies with its molecular weight as shown in table 2

Hence, this paper reveals that by a using simple method it is possible to identify all the impurities of acetaminophen in a finished product by a single injection. . the following figures shows that mass spectrum of each impurity of acetaminophen [figure-2 to figure 17]

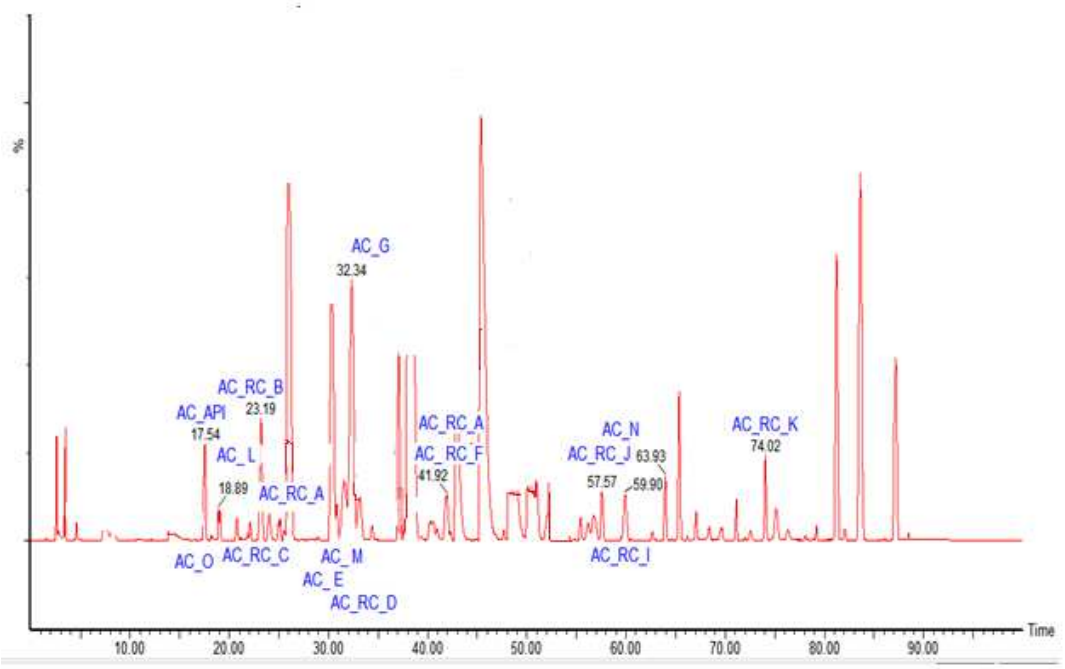

Figure 2 : TIC of all related substance of acetaminophen

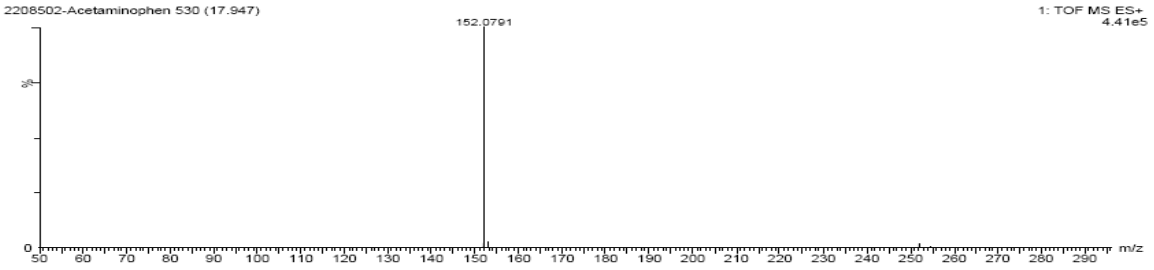

Figure 3. Mass spectrum of Acetaminophen API

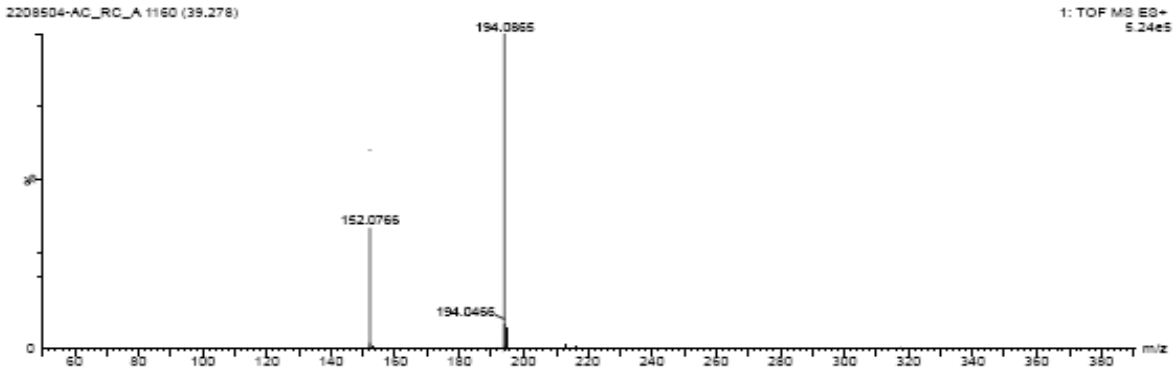

Figure 4. Mass spectrum of

Acetaminophen related compound A

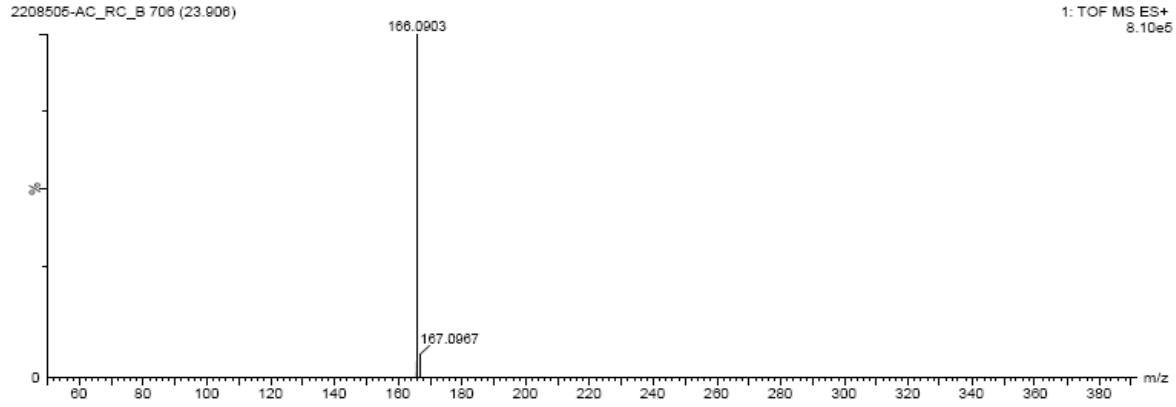

Figure 5. Mass spectrum of

Acetaminophen related compound B

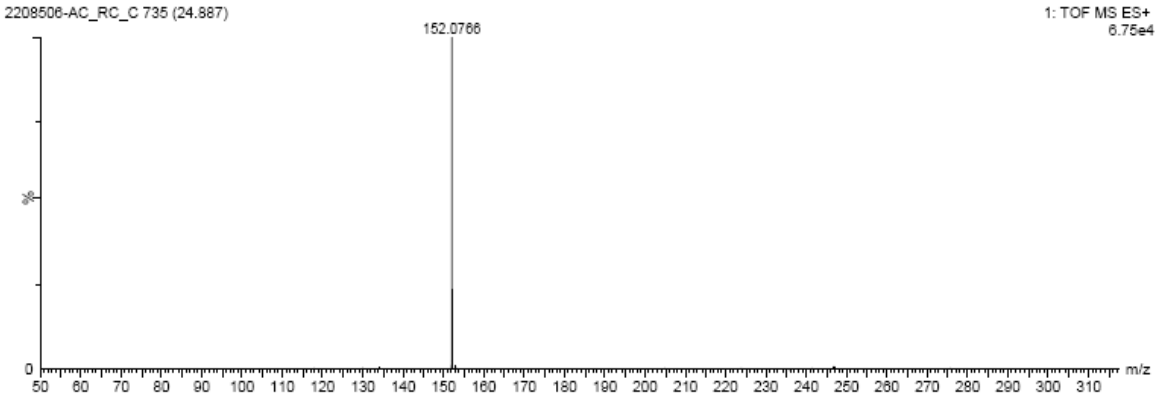

Figure 6.Mass spectrum of

Acetaminophen related compound C

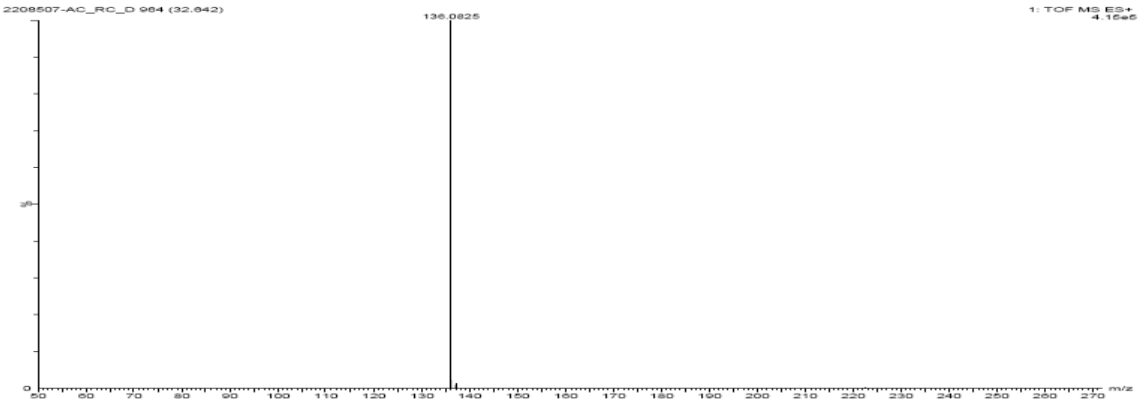

Figure 7. Mass spectrum of

Acetaminophen related compound D

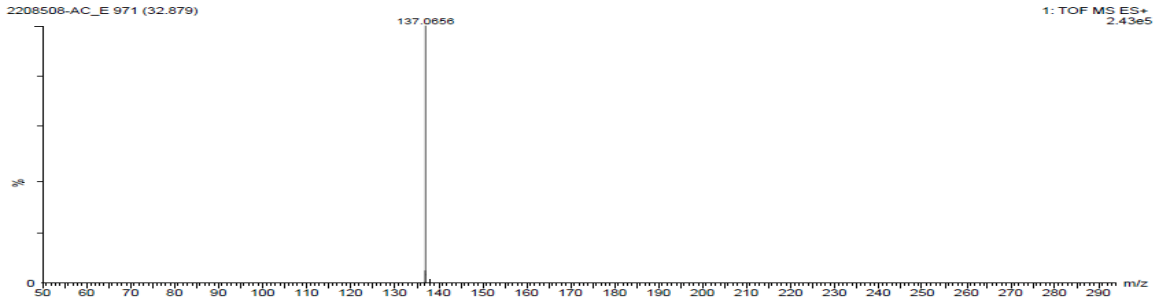

Figure 8. Mass spectrum of

Acetaminophen Impurity E

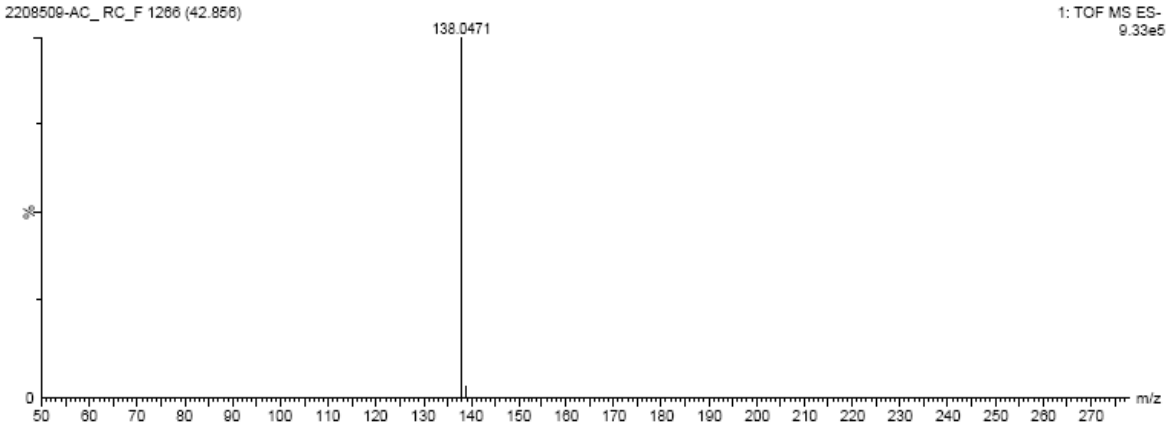

Figure 9. Mass spectrum of

Acetaminophen related compound F

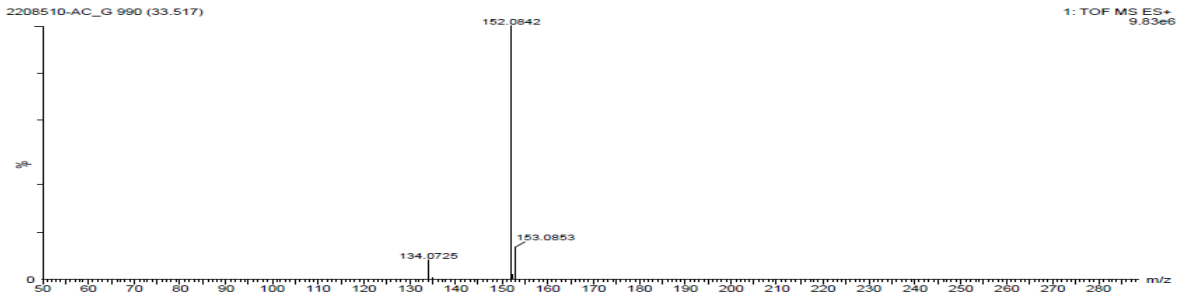

Figure 10. Mass spectrum of

Acetaminophen Impurity G

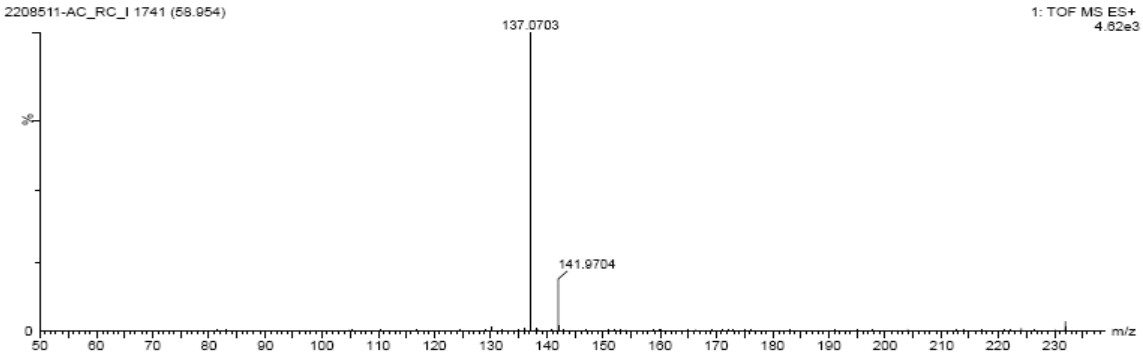

Figure 11. Mass spectrum of

Acetaminophen related compound I

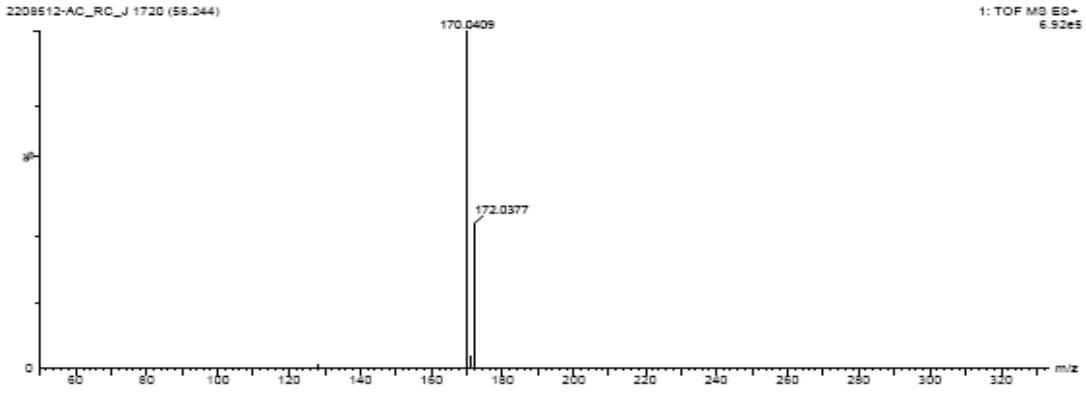

Figure 12. Mass spectrum of

Acetaminophen related compound J

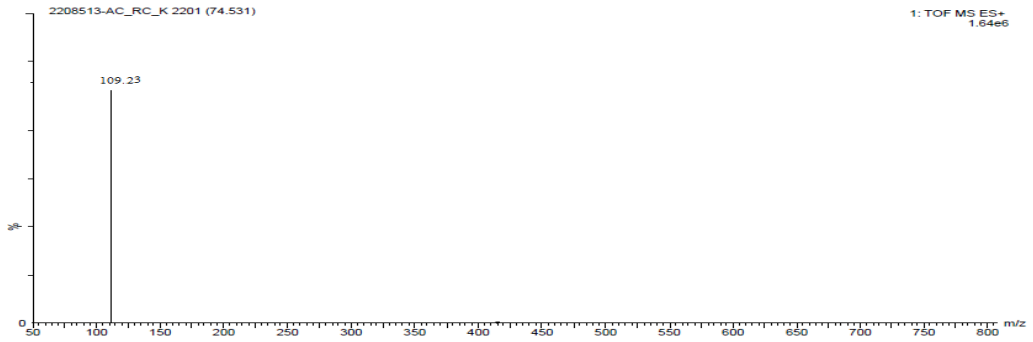

Figure 13. Mass spectrum of

Acetaminophen related compound K

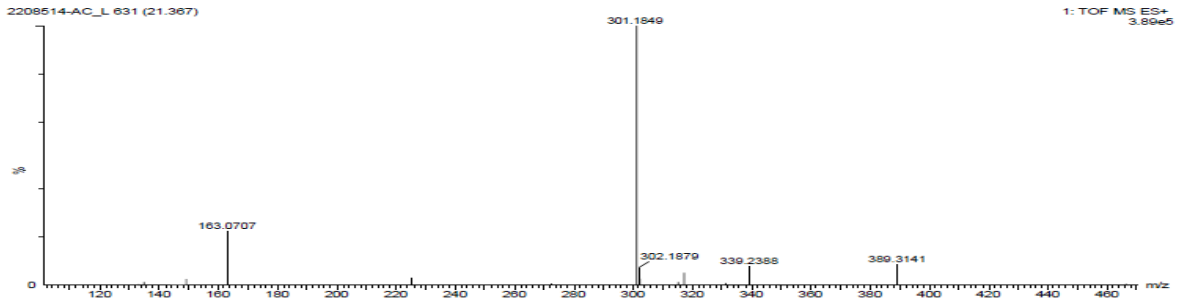

Figure 14. Mass spectrum of

Acetaminophen Impurity L

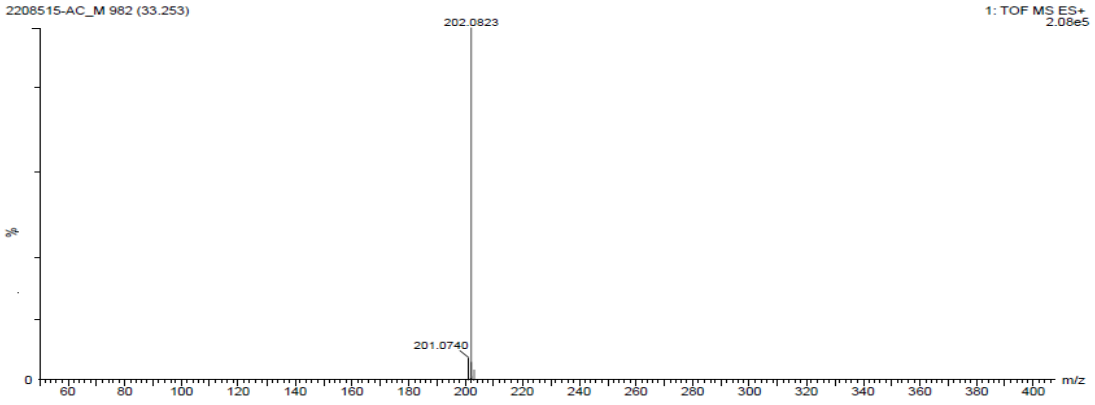

Figure 15. Mass spectrum of

Acetaminophen Impurity M

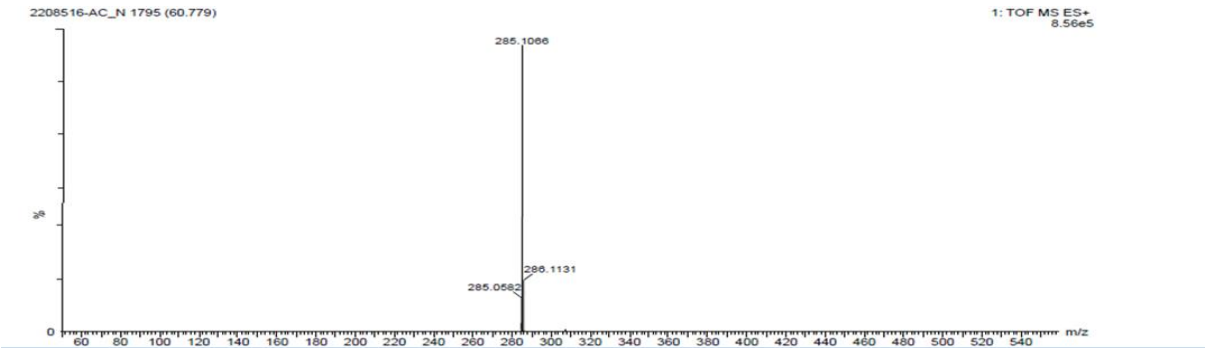

Figure 16. Mass spectrum of

Acetaminophen Impurity N

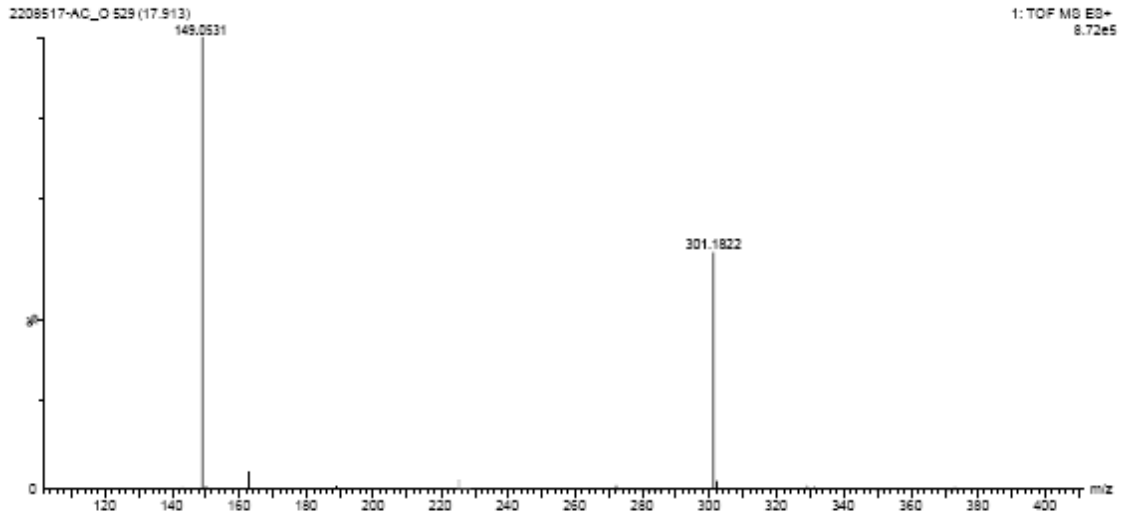

Figure 17. Mass spectrum of

Acetaminophen Impurity O

4. CONCLUSION

A single and simple method is developed for determination of impurities of acetaminophen along its Active ingredient in a marketed acetaminophen drug. Thereby **I concluded that in a single injection it's possible to identify acetaminophen and its related substance by LCMS (as shown in figure 2).**

5. Declarations

a. Ethics approval and consent to participate

Not applicable

b. Consent for publication

Not applicable

c. Availability of data and materials

Not applicable

d. Competing interests

Not applicable

e. Funding

Not applicable

f. Authors' contributions

Not applicable

6.REFERENCE

1. Simultaneous determination of paracetamol, propyphenazone, aspirin and caffeine in white wine samples by liquid chromatography-triple quadrupole tandem mass spectrometry DOI: https://doi.org/10.21203/rs.3.rs-2794814/v1.
2. Smart chemometrics-assisted spectrophotometric methods for efficient resolution and simultaneous determination of paracetamol, caffeine, drotaverine HCl along with three of their corresponding related impurities Samia A. Tawfk1*, Maha A. Hegazy1 Nariman A. El-Ragehy1 and Ghada A. Sedik1.
3. IMPURITY PROFILING OF PARACETAMOL DOSAGE FORMS USEDIN MAIDUGURI METROPOLIS Hassan Yusufi Braima, Abubakar Bab Akura Tijjani1and Samuel Cabiri Amo WJPR.
4. HPLC Separation of Acetaminophen and its Impurities Using a Mixed-mode Reversed-Phase/Cation Exchange Stationary Phase Octavian Ca˘linescu1, Irinel A. Badea1*, Luminita Vla˘descu1, Viorica Meltzer2 and Elena Pincu2. Journal of Chromatographic Science 2012;50:335–342
5. Analytical techniques for the determination of acetaminophen: A review Hanieh Moontasri, Patricia BC Forbes Track Trends in Analytical Chemistry 108, 122-134, 2018

7.Acknowledgement :

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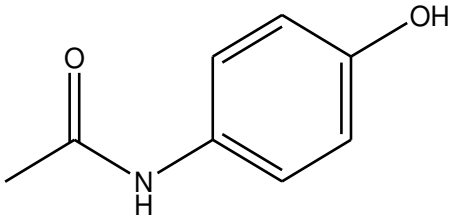
Figures



ACETAMINOPHEN

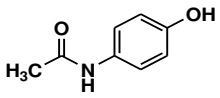
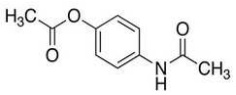
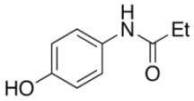
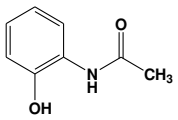
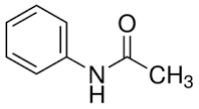
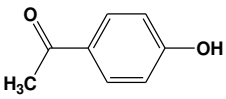
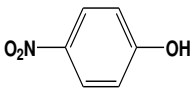
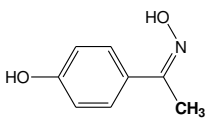
Figure 1: Structure of Acetaminophen

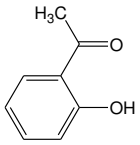
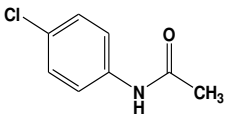
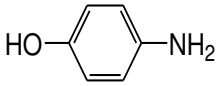
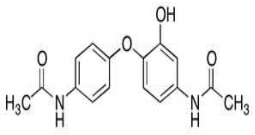
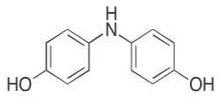
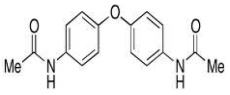
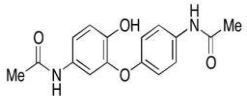
Tables

Time in min	Flow rate per min	Mobile phase A	Mobile phase B
Initial	0.600	100.0	0.0
10.00	0.600	100.0	0.0
15.00	0.600	90.0	10.0
40.00	0.600	80.0	20.0
60.00	0.600	70.0	30.0
90.00	0.600	40.0	60.0
91.00	0.600	100.0	0.0
110.00	0.600	100.0	0.0

Table 1. Gradient Programme In LC

Table 2: List Of Acetaminophen Impurities

Sl. No.	Name of the compound	Code	IUPAC name	Structure of the molecule	Molecular weight
1	Acetaminophen API	AC_API	4'-hydroxy acetanilide		151.2
2	Acetaminophen related compound A	AC_RC_A	4-(Acetylamino) phenylacetate		193.2
3	Acetaminophen related compound B	AC_RC_B	N-(4-hydroxy phenyl)propan amide		165.2
4	Acetaminophen related compound C	AC_RC_C	N-(2-Phydroxy phenyl)acetam ide		151.2
5	Acetaminophen related compound D	AC_RC_D	**N-Phenyl-acetamide**		135.2
6	Acetaminophen Impurity E	AC_E	4′-Hydroxy acetophenone		136.2
7	Acetaminophen related compound F	AC_RC_F	4-Nitrophenol		139.2
8	Acetaminophen Impurity G	AC_G	1-(4-Hydroxypheny l) ethanone oxime		151.2

9	Acetaminophen related compound I	AC_RC_I	1-(2-Hydroxy phenyl)ethano ne		136.2
10	Acetaminophen related compound J	AC_RC_J	**N-(4-Chlorophenyl acetamide**		169.6
11	Acetaminophen related compound K	AC_RC_K	4-Aminophenol		109.1
12	Acetaminophen Impurity L	AC_L	N-[4-[4-Acetamido -2-hydroxypheno xy) phenyl]acetam ide		300.3
131	Acetaminophen Impurity M	AC_M	4-(4-hydroxy phenylamino) phenol		201.2
114	Acetaminophen Impurity N	AC_N	Bis(p-acetylamino phenyl) ether		284.3
15	Acetaminophen Impurity O	AC_O	2-Hydroxy-4',5-diacetamido-diphenyl ether		300.3

Abbreviations

- LCMS- Liquid Chromatography – Mass Spectrometry
- OTC – Over the Counter
- HPLC – High performance liquid chromatography
- ESI – electron spray ionisation
- LC – Liquid chromatography
- TIC – Total ionic current
- V- voltage
- GC- Gas Chromatography
- NMR- Nuclear Magnetic Resonance
- CE- capillary Electrophoresis
- MS- Mass spectrometry